Реагенты для клеточного анализа

Технические характеристики

Виды товаров: реагенты и наборы для апоптоза, реагенты для пролиферации клеток, реагенты для флуоресцентного окрашивания клеток, для окрашивания стареющих клеток реагенты клеточной сигнализации, реагенты для трансфекции лентивируса, реагенты для трансфекции клеток, колонка для разделения экзосом и др.

По вопросам продаж и поддержки обращайтесь:

Алматы (7273)495-231
Ангарск (3955)60-70-56
Архангельск (8182)63-90-72
Астрахань (8512)99-46-04
Барнаул (3852)73-04-60
Белгород (4722)40-23-64
Благовещенск (4162)22-76-07
Брянск (4832)59-03-52
Владивосток (423)249-28-31
Владикавказ (8672)28-90-48
Владимир (4922)49-43-18
Волгоград (844)278-03-48
Вологда (8172)26-41-59
Воронеж (473)204-51-73
Екатеринбург (343)384-55-89
Иваново (4932)77-34-06
Ижевск (3412)26-03-58
Иркутск (395)279-98-46
Казань (843)206-01-48
Калининград (4012)72-03-81
Калуга (4842)92-23-67
Кемерово (3842)65-04-62
Киров (8332)68-02-04
Коломна (4966)23-41-49
Кострома (4942)77-07-48
Краснодар (861)203-40-90
Красноярск (391)204-63-61
Курск (4712)77-13-04
Курган (3522)50-90-47
Липецк (4742)52-20-81
Магнитогорск (3519)55-03-13
Москва (495)268-04-70
Мурманск (8152)59-64-93
Набережные Челны (8552)20-53-41
Нижний Новгород (831)429-08-12
Новокузнецк (3843)20-46-81
Ноябрьск (3496)41-32-12
Новосибирск (383)227-86-73
Омск (3812)21-46-40
Орел (4862)44-53-42
Оренбург (3532)37-68-04
Пенза (8412)22-31-16
Петрозаводск (8142)55-98-37
Псков (8112)59-10-37
Пермь (342)205-81-47
Ростов-на-Дону (863)308-18-15
Рязань (4912)46-61-64
Самара (846)206-03-16
Санкт-Петербург (812)309-46-40
Саратов (845)249-38-78
Севастополь (8692)22-31-93
Саранск (8342)22-96-24
Симферополь (3652)67-13-56
Смоленск (4812)29-41-54
Сочи (862)225-72-31
Ставрополь (8652)20-65-13
Сургут (3462)77-98-35
Сыктывкар (8212)25-95-17
Тамбов (4752)50-40-97
Тверь (4822)63-31-35
Тольятти (8482)63-91-07
Томск (3822)98-41-53
Тула (4872)33-79-87
Тюмень (3452)66-21-18
Ульяновск (8422)24-23-59
Улан-Удэ (3012)59-97-51
Уфа (347)229-48-12
Хабаровск (4212)92-98-04
Чебоксары (8352)28-53-07
Челябинск (351)202-03-61
Череповец (8202)49-02-64
Чита (3022)38-34-83
Якутск (4112)23-90-97
Ярославль (4852)69-52-93

Россия +7(495)268-04-70 Казахстан +7(7172)727-132 Киргизия +996(312)96-26-47

эл.почта: sih@nt-rt.ru || сайт: https://servicebio.nt-rt.ru/

Servicebio® Cell Cycle and Apoptosis Detection Kit

Cat. #: G1700-50T

Product Information

Product Name	Cat. No	Spec.
Cell Cycle and Apoptosis Detection Kit	G1700-50T	50 T

Product Description/Introduction

Cell cycle refers to the whole process of the cell from the completion of one division to the end of the next division, which is mainly divided into two stages: intermitotic phase and mitotic phase (M phase). The intermitotic phase is mainly composed of the early phase of DNA synthesis (G1 phase), DNA synthesis (S phase) and the late phase of DNA synthesis (G2 phase). The sequence of the whole cell cycle can be expressed as G1→S→G2→M. First of all, in G1 phase, cells mainly synthesize RNA and proteins to prepare materials and energy for cells to enter S phase. Then it enters S phase: the cells begin to synthesize DNA and some histones and other substances, and the cell DNA content begins to increase. Finally, the G2 phase: at this time, the DNA content of the cell has become twice that of the G1 phase, and has stopped DNA replication to enter the mitotic phase to do a lot of protein and other material synthesis; If the cell is in the G0 (Cells temporarily stop dividing, differentiating, and quiescence)/G1 phase, the DNA content of the cell is 1N; So the DNA content of the cell in G2 phase is 2N; The DNA content of S-phase cells between G1 and G2 is 1N to 2N. In apoptotic cells, the nucleus will be concentrated and DNA fragmentation will occur, leading to the loss of part of genomic DNA fragments, so the DNA content is less than 1N, and the so-called sub-G1 peak, namely the apoptotic cell peak, appears on the fluorogram of the flow cytometry. Therefore, the cell cycle and state can be judged according to the content of cell DNA.

Apoptosis can also be detected by observing changes in cell light scattering by flow cytometry. Apoptosis occurs when cells produce apoptotic bodies as a result of cytoplasmic and chromatin condensation and nuclear fragmentation. Chromatin shrinks and cell density increases in pre-apoptosis stage. In the late stage of apoptosis, cells produce apoptotic bodies, and the light scattering of cells changes.

The Cell Cycle and Apoptosis Analysis Kit use the classic Propidium staining method to detect and analyze Cell Cycle and Apoptosis. Propidium iodide is able to embed in double-stranded DNA and cause it to fluoresce. The cycle and state of a cell can be distinguished by the characteristic that the fluorescence intensity is proportional to the content of double-stranded DNA and the regular change of DNA content in different cell cycles. This kit can be used for cell cycle and apoptosis detection of tissue cells, adherent or suspended cells (if used for cell cycle and apoptosis detection of tissue, the tissue must be digested into a single cell state before detection).

Storage and Shipping Conditions

Ship with wet ice ; Store at -20℃ away from light, dyeing buffer store at 4℃, valid for 12 months.

Product Components

Component Number	Component	G1700-50T
G1700-1	PI Staining Solution (50×)	500 μL

G1700-2	RNase A (50×)	500 μL
G1700-3	Staining Buffer	25 mL
Manual		1 pc

Note

1. Cell culture medium containing serum.
2. Trypsin digestion solution (G4001 is recommended).
3. PBS buffer (G4202 is recommended).
4. 75% ethanol.

Product Protocol/Procedures

1. Preparation of Cell Samples (the number of cells is controlled at 1×10^5~1×10^6)

a) **For adherent cells:** remove the culture medium, add trypsin digestion solution to digest the cells, and observe that the cells become round and loose under the microscope. Add the appropriate amount of cell medium contains serum to terminate the digestion, gently blow the cells apart and make a suspension of the cells. transfer the suspension to a centrifuge tube, centrifuge at 1000 x g for 3-5 min, discard the supernatant and retain the cell precipitation. Then wash the cell precipitation 1-2 times with pre-cooled PBS buffer, and discard the supernatant by centrifugation in the same way to retain the cell precipitation.

b) **For the suspended cells:** transfer the cells to a centrifuge tube, centrifuge at 1000 x g for 3-5 min, discard the supernatant, and retain the cell precipitation; Wash the cell precipitation 1-2 times with pre-cooled PBS buffer, and discard the supernatant by centrifugation in the same way to retain the cell precipitation.

c) **For tissue cells:** Cut the tissue into small pieces as much as possible, digest the pieces with digestive enzymes such as trypsin and collagenase according to the source of the tissue, and filter the tissue through a 100-300 mesh sieve to obtain a single cell suspension; transfer the filtered cell suspension to a centrifuge tube, centrifuge at 1000 x g for 3-5 min, discard the supernatant and retain the cell precipitation; then wash the cell precipitate 1-2 times with pre-cooled PBS buffer, discard the supernatant by centrifuge in the same way to retain the cell precipitation.

2. Fixation of Cell Samples

a) Add 1 mL of 75% pre-cooled ethanol on ice to the collected cell precipitation sample, and gently blow the cells to make them thoroughly mixed.

b) Cells are fixed at 4°C for 30 min or longer (usually 2 h or more is better for staining, 12-24 h may be better to improve staining effect).

c) After fixed for a certain time, the cells are centrifuged at 1000 x g for 3-5 min to remove the ethanol fixing solution and retain the cell precipitation.

d) Tap the bottom of the centrifuge tube to disperse the cells, resuspend and wash the cells with PBS buffer, centrifuge at 1000 x g for 3-5 min, discard the supernatant and collect the cell precipitation.

3. Preparation and Staining of Working Solution

a) According to the following table, the dyeing solution can be prepared out of light, and the amount can be increased or decreased in equal proportion according to the use requirements

(the prepared dyeing solution can be stored at 4℃ in a short time, please use it within the same day).

	1 Sample	5 Samples	10 Samples
Staining Buffer	480 μL	2.4 mL	4.8 mL
PI Stain (50×)	10 μL	50 μL	100 μL
RNaseA (50×)	10 μL	50 μL	100 μL
Total Volume	500 μL	2.5 mL	5 mL

b) Tap the bottom of the centrifuge tube to disperse the cells precipitation in Step 2.4, then add 500 μL of the prepared staining working solution, and gently blow to disperse the cells and mix with the staining working solution.

c) Incubate at 37℃ for 30 min in the dark, use flow cytometry for detection.

4. Flow Detection and Analysis

A flow cytometer is used to detect red fluorescence at an excitation wavelength of 488 nm, together with light scattering. Cell DNA content analysis and light scattering analysis are performed using appropriate analysis software.

Note

1. Fluorescent dyes are subject to fluorescence quenching and should be protected from light during use and storage.
2. It is recommended to synchronise the cells before the experiment to avoid large reproducibility differences caused by different cell cycles.
3. The planting density of experimental cells should not be too high or too low to prevent contact inhibition or density dependence.
4. Protect from direct contact with humans or inhalation when handling PI staining solution.
5. For your safety and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® Fluorescein (FITC) Tunel Cell Apoptosis Detection Kit

Cat #: G1501

Product Information

Product Name	Cat. No	Spec.
Fluorescein (FITC) Tunel Cell Apoptosis Detection Kit	G1501-50T	50 T
	G1501-100T	100 T

Product Description/Introduction

The breakage of chromosomal DNA in apoptosis is a gradual process. Chromosomal DNA is first degraded into large fragments of 50-300 KB by endogenous nuclease hydrolase, and then about 30% of chromosomal DNA is randomly cut between nucleosome units under the action of Ca^{2+} and Mg^{2+} dependent nucleic acid endonuclease to form 180-200 bp nucleosome DNA polymers. Therefore, in late apoptosis, DNA is degraded into 180-200 bp fragments, and a large number of 3'-OH terminals are exposed on the broken genomic DNA. Terminal deoxyribonucleotidyl transferase (TDT) is a template independent DNA polymerase, which can catalyze the binding of deoxynucleotides to the 3'-OH terminal of broken DNA molecules. Therefore, TUNEL (TDT mediated dUTP nick end labeling) cell apoptosis detection kit can be used to detect the breakage of nuclear DNA in tissue cells during the late apoptosis. It is based on the incorporation of fluorescein-labelled dUTP (FITC-12-dUTP) at the exposed 3´-OH ends of genomic DNA breaks by TdT enzymes, which can be detected by fluorescence microscopy or flow cytometry (FITC excitation 495 nm, emission 521 nm). This kit is suitable for apoptosis detection of paraffin tissue sections, frozen tissue sections, cell crawling, cell smears.

Storage and Shipping Conditions

Ship with wet ice; This kit store at -20°C, FITC-12-dUTP Labeling Mix store at -20°C protect from light and valid for 12 months.

Product Components

Component Number	Component	G1501-50T	G1501-100T
G1501-1	Recombinant TdT Enzyme	50 μL	2×50 μL
G1501-2	FITC-12-dUTP Labeling Mix	250 μL	2×250 μL
G1501-3	Equilibration Buffer	5×1 mL	10×1 mL
G1501-4	Proteinase K (200 μg/mL)	1 mL	2×1 mL
Manual		1 pc	

Product Preparation

1. PBS phosphate buffer (recommended G0002 or G4202).
2. Fixative solution: 4% paraformaldehyde dissolved in PBS or other buffer systems, pH 7.4 (recommended G1101).

3. Membrane breaking liquid：0.1%-0.5% Triton X-100 (recommended G1204).
4. If you need to dye the nucleus, you need to bring your own DAPI (2 μg/mL)or PI (1 μg/mL) (recommended G1012,G1021).
5. For positive control experiments, DNase I is required (recommended G3342).
6. If using a flow cytometer, bring your own PI staining solution (recommended G1021)and RNase A (DNase free).
7. For your safety and health, please wear safety glasses, gloves, or protective clothing.

Product Protocol/Procedures

1. Sample Preparation

A. Paraffin Tissue Sections

1) Soak paraffin tissue sections in BioDewax and Clear Solution (G1128) for 5-10 min at room temperature and repeat 3 times; Then in anhydrous ethanol for 5 min and repeated twice; Finally in gradient ethanol (85%, 75%, and double distilled water) once for 5 min each time.
2) Wash slices gently with PBS and remove the excess liquid around the sample. Use a histochemical pen to draw a small circle 2-3 mm apart along the peripheral contour of the tissue to facilitate downstream permeability treatment and balance labelling. During the experiment, do not let the sample dry, and put the treated sample in the wet box to keep the sample moist.
3) Prepare Proteinase K working solution: dilute Proteinase K (200 μg/ml) stock solution with PBS at a ratio of 1:9 to a final concentration of 20 μg/mL.
4) Add 100 μL of Proteinase K working solution to each sample, completely cover the tissue and incubate at 37 ℃ for 20 min.

Note: Proteinase K contributes to the permeation of staining reagents in the subsequent steps of the tissue and cells. Incubate for too long or too short will affect the efficiency of the subsequent labelling. For better results, the incubation time of Proteinase K can be optimized.

5) Soak and clean the sample with PBS solution for 3 times, each time for 5 min (Proteinase K needs to be cleaned, otherwise it will interfere with the subsequent marking reaction). keep the sample moist in the wet box after treatment.
6) (optional steps) remove the excess liquid from the sample, add an appropriate amount of membrane breaking liquid to the tissue, fully infiltrate the tissue, and treat at room temperature for 20 min; After the membrane breaking treatment is completed, wash the sample three times with PBS solution for 5 min each time; keep the sample moist after treatment in a wet box.

B. Frozen Tissue Sections

1) Immerse the slides in 4% paraformaldehyde solution (dissolved in PBS) for fixation, and incubate at room temperature for 10-15 min.
2) Remove the film from the fixation solution and let it dry naturally in a fume hood.
3) Wash the slide in pure water or PBS to remove residual fixation solution from the slide.
4) Use a histochemical pen to draw a small circle 2-3 mm apart along the peripheral contour of the tissue to facilitate downstream permeability treatment and balance labelling. During the experiment, do not let the sample dry, and put the treated sample in the wet box to keep moist.
5) Prepare Proteinase K working solution: dilute Proteinase K (200 μg/ml) stock solution with PBS at a ratio of 1:9 to a final concentration of 20 μg/mL.
6) Add 100 μL of Proteinase K working solution to each sample, completely cover the tissue and incubate at 37 ℃ for 10 min.

Note: Proteinase K contributes to the permeation of staining reagents in the subsequent steps of the tissue and cells. Incubate for too long or too short will affect the efficiency of the subsequent labelling. For better results, the incubation time of Proteinase K can be optimized.

7) Rinse the sample with PBS solution for 2-3 times to remove the excess liquid (Proteinase K needs to be cleaned, otherwise it will interfere with the subsequent marking reaction). keep the sample moist in the wet box after treatment.
8) (optional steps) add an appropriate amount of membrane breaking liquid to the tissue, fully infiltrate the tissue, and treat it at room temperature for 20 min. After completion of the membrane breaking treatment, wash the sample with PBS solution to remove the excess liquid. keep the sample moist after treatment in a wet box.

C. Cell Crawling

1) Adherent cells are cultured on lab-Tek (Chamber Slides). After apoptosis induction treatment, the slides are gently rinsed twice with PBS.
2) Add an appropriate amount of 4% paraformaldehyde solution (dissolved in PBS) to each slide chamber for fixation, and incubate at room temperature for 20 min.
3) Remove the fixation solution and wash three times with PBS, each time for 5 min.
4) Immerse samples in the membrane breaking solution and incubate at room temperature for 5 min for permeabilisation.(Note: It is recommended to use 2-20 μg/mL Proteinase K working solution for digestion and treatment at 37℃ for about 10 min to adjust the state of cells. If the cells are easy to fall off, it is recommended to choose the membrane breaking solution treatment)
5) Immerse and clean the sample in an open beaker containing PBS solution for 2-3 times.
6) Gently remove excess liquid and carefully drain the liquid around the sample on the slide with filter paper. Keep the sample moist after treatment in a wet box.

D. Cell Smear

1) Resuspend cells in PBS at a concentration of approximately 2×10^7 cells/mL, pipette 50-100 μL of cell suspension onto an anti-defossing slide, and gently spread the cell suspension using a clean slide.
2) Immerse the slides in a staining jar filled with 4% paraformaldehyde freshly formulated in PBS, fix the cells, and leave at 4 °C for 25 min.
3) Immerse the slide in PBS, place it at room temperature for 5 min, and repeat once.
4) Gently remove the excess liquid, and carefully drain the excess liquid around the sample on the slide with filter paper, draw a small circle along the outer contour of the cell with a histochemical pen to facilitate downstream permeability processing and balance labeling operations, and do not allow the sample to dry during the experiment.
5) Immerse samples in the membrane breaking solution and incubate at room temperature for 5 min for permeabilisation.(Note: It is recommended to use 2-20 μg/mL Proteinase K working solution for digestion and treatment at 37℃ for about 10 min to adjust the state of cells. If the cells are easy to fall off, it is recommended to choose the membrane breaking solution treatment)
6) Immerse and clean the sample in an open beaker containing PBS solution for 2-3 times.
7) Gently remove excess liquid and carefully drain the liquid around the sample on the slide with filter paper. Keep the sample moist after treatment in a wet box.

2. DNase I Treatment Positive Control Experiment (Optional Steps)

After the sample permeability treatment, treat the sample with DNase I (recommended G3342) to prepare the positive control.

1) Add 100 μL of 1× DNase I buffer (preparation method: mix well 10 μL of 10× DNase I buffer and 90 μL of deionized water) to the permeable sample and incubate at room temperature for 5 min.
2) Gently remove excess liquid and add 100 μL of working solution containing DNase I (20 U/ml) (preparation method: mix well 10 μL of 10× DNase I buffer, 2 μL of DNase I and 88 μL of deionized water), incubate at room temperature for 10 min.
3) Gently remove the excess liquid, and thoroughly wash the slide 3-4 times in the staining tank with PBS;

(**Note**: the positive control slide must use a separate staining cylinder, otherwise the residual DNase I on the positive control slide may introduce a high background on the experimental slide).

3. Marking and Testing

1) Equilibration: Add 50 μL of Equilibration Buffer per sample to cover the sample area and incubate at room temperature for 10 min.
2) Labeling solution preparation: thaw FITC-12-dUTP Labeling Mix and Equilibration Buffer on ice and mix sufficient TdT incubation buffer according to the ratio of Recombinant TdT enzyme: FITC-12-dUTP Labeling Mix: Equilibration Buffer=1 μL: 5 μL: 50 μL (1: 5: 50). The volume of reagents used in specific experiments can be adjusted in an appropriate equal proportion according to the size of the slide.
3) Negative control system: Prepare a control TdT incubation buffer without Recombinant TdT enzyme and replace it with ddH_2O.
4) Labeling: remove the equilibration Buffer and then add 56 μL of TdT incubation buffer to each tissue sample and incubate at 37 °C for 1 h; Note that the slides should not be dried and protected from light.
5) Immediately wash the tissue samples with PBS four times for 5 min each.
6) Gently wipe off the PBS solution around the sample with filter paper.
7) Nuclear staining: Stain the samples in a staining vat and immerse the slides with PI solution or DAPI solution (freshly formulated and diluted with PBS) in the dark at room temperature for 8 min.
8) Sealing: After staining, wash the sample three times with PBS for 5 min each, then gently remove the excess liquid and sealing the slide with the anti-fluorescence quenching sealer (recommended G1401)).
9) Microscopy: Immediately analyze the sample under a fluorescence microscope, the slides are carefully protected from light, PI/DAPI can stain both apoptotic and not apoptotic cells red/blue, green fluorescence localized by FITC-12-dUTP incorporation only in the nuclei of apoptotic cells.

4. Suspension Cells are Detected Using Flow Cytometry

1) The cells to be detected are washed twice with PBS, centrifuge (500 x g) at 4°C and resuspended in 500 μL of PBS.
2) Fixation: Add 5 mL of 1% paraformaldehyde solution prepared with PBS to the sample, fix the cells, and leave on ice for 20 min.
3) Cells are centrifuged at 4 °C, 300 x g for 10 min, supernatanted and resuspended twice with 5 mL of PBS, and finally resuspended with 500 μL of PBS.
4) Permeability: Add 5 mL of pre-cooled 70% ethanol on ice to the sample and incubate at -20°C for 4 h to permeabilise the cells.

(**Note**: Cells can also be permeated with membrane breaking solution at room temperature for 5 min)

5) Cells are resuspended with 5 mL of PBS after centrifugation at 300 x g for 10 min, and resuspended with 1 mL of PBS after centrifugation again.

6) Equilibration: Transfer approximately 2×10^6 cells to a 1.5 mL microcentrifuge tube, centrifuge at 300 x g for 10 min, discard the supernatant and resuspend with 80 μL of Equilibration Buffer, incubate at room temperature for 5 min.

7) Labeling solution preparation: thaw FITC-12-dUTP Labeling Mix and Equilibration Buffer on ice and mix sufficient TdT incubation buffer according to the ratio of Recombinant TdT enzyme: FITC-12-dUTP Labeling Mix: Equilibration Buffer=1 μL: 5 μL: 50 μL (1: 5: 50) for all experiments and optional positive control reactions.

8) Labeling: Cells are centrifuged at 300 x g for 10 min, discard the supernatant and resuspend the precipitate in 56 μL of TdT incubation buffer, incubate at 37°C for 1 h, protect from light. Gently resuspend cells with a micropipette every 15 min.

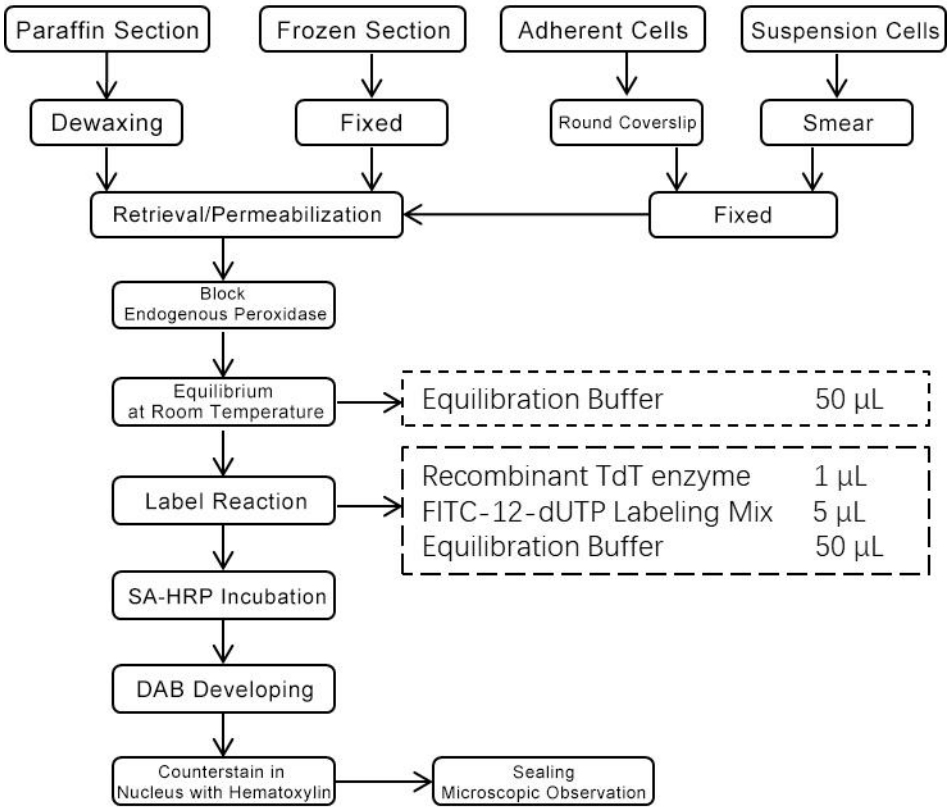
9) After the reaction is complete, add 1 mL of 20 mM EDTA to terminate the reaction and mix gently with a micropipette.

10) Centrifuge at 300 x g for 10 min, discard the supernatant and resuspend the precipitate in 1 mL of membrane breaking solution containing 5 mg/mL BSA, repeat wash twice.

11) Nuclear staining: Centrifuge at 300 x g for 10 min, discard the supernatant and resuspend the cell precipitate in 0.5 mL of PI solution containing 250 μg of RNase A without DNAase, incubate the cells in the dark at room temperature for 30 min.

12) Hands-on detection: Flow cytometry analyzes cells, PI can dye both apoptotic and unpoptoptotic cells red/blue, and only in the apoptosis nucleus does FITC-12-dUTP incorporate and locate the green fluorescence.

5. Experimental Process Diagram



Servicebio® TMR (Red) Tunel Cell Apoptosis Detection Kit

Cat #: G1502

Product Information

Product Name	Cat. No.	Spec.
TMR (Red) Tunel Cell Apoptosis Detection Kit	G1502-50T	50 T
	G1502-100T	100 T

Product Description/Introduction

The breakage of chromosomal DNA in apoptosis is a gradual process. Chromosomal DNA is first degraded into large fragments of 50-300 KB by endogenous nuclease hydrolase, and then about 30% of chromosomal DNA is randomly cut between nucleosome units under the action of Ca^{2+} and Mg^{2+} dependent nucleic acid endonuclease to form 180-200 bp nucleosome DNA polymers. Therefore, in late apoptosis, DNA is degraded into 180-200 bp fragments, and a large number of 3'-OH terminals are exposed on the broken genomic DNA. Terminal deoxyribonucleotidyl transferase (TDT) is a template independent DNA polymerase, which can catalyze the binding of deoxynucleotides to the 3'-OH terminal of broken DNA molecules. Therefore, TUNEL (TDT mediated dUTP nick end labeling) cell apoptosis detection kit can be used to detect the breakage of nuclear DNA in tissue cells during the late apoptosis. The principle is that in the presence of TdTase, the 3´-OH ends exposed at genomic DNA breaks are doped with Tetramethyl-Rhodamine-5-dUTP (TMR-5-dUTP), which can be detected by fluorescence microscopy or flow cytometry (TMR excitation 551 nm, emission 575 nm). The kit has a wide range of applications and is suitable for apoptosis detection in paraffin tissue sections, frozen tissue sections, cell crawls and cell smears.

Storage and Shipping Conditions

Ship with wet ice; This kit store at -20 °C, TMR-5-dUTP Labeling Mix store at -20 °C protect from light and valid for 12 months.

Product Components

Component Number	Component	G1502-50T	G1502-100T
G1502-1	Recombinant TdT Enzyme	50 μL	2×50 μL
G1502-2	TMR-5-dUTP Labeling Mix	250 μL	2×250 μL
G1502-3	Equilibration Buffer	5×1 mL	10×1 mL
G1502-4	Proteinase K (200 μg/mL)	1 mL	2×1 mL
Manual		1 pc	

Product Preparation

1. PBS phosphate buffer (recommended G0002 or G4202).
2. Fixative solution: 4% paraformaldehyde dissolved in PBS or other buffer systems, pH 7.4 (recommended G1101).
3. Membrane breaking liquid: 0.1%-0.5% Triton X-100 (recommended G1204).

4. If you need to dye the nucleus, you need to bring your own DAPI (2 μg/mL)or PI (1 μg/mL) (recommended G1012,G1021).
5. For positive control experiments, DNase I is required (recommended G3342).
6. If using a flow cytometer, bring your own PI staining solution (recommended G1021) and RNase A (DNase free) (recommended G3405).
7. For your safety and health, please wear safety glasses, gloves, or protective clothing.

Assay Protocol / Procedures

1. **Sample Preparation**

a) **Paraffin Tissue Sections**

i. Soak paraffin tissue sections in BioDewax and Clear Solution (G1128) for 5-10 min at room temperature and repeat 3 times; Then in anhydrous ethanol for 5 min and repeated twice; Finally in gradient ethanol (85%, 75%, and double distilled water) once for 5 min each time.

ii. Wash slices gently with PBS and remove the excess liquid around the sample. Use a PAP Pen to draw a small circle 2-3 mm apart along the peripheral contour of the tissue to facilitate downstream permeability treatment and balance labelling. During the experiment, do not let the sample dry, and put the treated sample in the wet box to keep the sample moist.

iii. Prepare Proteinase K working solution: dilute Proteinase K (200 μg/ml) stock solution with PBS at a ratio of 1:9 to a final concentration of 20 μg/mL.

iv. Add 100 μL of Proteinase K working solution to each sample, completely cover the tissue and incubate at 37 ℃ for 20 min.

Note: Proteinase K contributes to the permeation of staining reagents in the subsequent steps of the tissue and cells. Incubate for too long or too short will affect the efficiency of the subsequent labelling. For better results, the incubation time of Proteinase K can be optimized.

v. Soak and clean the sample with PBS solution for 3 times, each time for 5 min (Proteinase K needs to be cleaned, otherwise it will interfere with the subsequent marking reaction). keep the sample moist in the wet box after treatment.

vi. (optional steps) remove the excess liquid from the sample, add an appropriate amount of membrane breaking liquid to the tissue, fully infiltrate the tissue, and treat at room temperature for 20 min; After the membrane breaking treatment is completed, wash the sample three times with PBS solution for 5 min each time; keep the sample moist after treatment in a wet box.

b) Frozen Tissue Sections

i. Immerse the slides in 4% paraformaldehyde solution (dissolved in PBS) for fixation, and incubate at room temperature for 10-15 min.

ii. Remove the slides from the fixation solution and let it dry naturally in a fume hood.

iii. Wash the slides in pure water or PBS to remove residual fixation solution from the slides.

iv. Use a PAP Pen to draw a small circle 2-3 mm apart along the peripheral contour of the tissue to facilitate downstream permeability treatment and balance labelling. During the experiment, do not let the sample dry, and put the treated sample in the wet box to keep moist.

v. Prepare Proteinase K working solution: dilute Proteinase K (200 μg/ml) stock solution with PBS at a ratio of 1:9 to a final concentration of 20 μg/mL.

vi. Add 100 μL of Proteinase K working solution to each sample, completely cover the tissue and incubate at 37℃ for 10 min.

Note: Proteinase K contributes to the permeation of staining reagents in the subsequent steps of the tissue and cells. Incubate for too long or too short will affect the efficiency of the subsequent labelling. For better results, the incubation time of Proteinase K can be optimized.

vii. Rinse the sample with PBS solution for 2-3 times to remove the excess liquid (Proteinase K needs to be cleaned, otherwise it will interfere with the subsequent marking reaction). keep the sample moist in the wet box after treatment.

viii. (optional steps) add an appropriate amount of membrane breaking liquid to the tissue, fully infiltrate the tissue, and treat it at room temperature for 20 min. After completion of the membrane breaking treatment, wash the sample with PBS solution to remove the excess liquid. keep the sample moist after treatment in a wet box.

c) Cell Crawling

i. Adherent cells are cultured on lab-Tek (Chamber Slides). After apoptosis induction treatment, the slides are gently rinsed twice with PBS.

ii. Add an appropriate amount of 4% paraformaldehyde solution (dissolved in PBS) to each slide chamber for fixation, and incubate at room temperature for 20 min.

iii. Remove the fixation solution and wash three times with PBS, each time for 5 min.

iv. Immerse samples in the membrane breaking solution and incubate at room temperature for 5 min for permeabilization (Note: It is recommended to use 2-20 μg/mL of Proteinase K working solution for digestion, and treat at 37℃ for about 10 min, depending on the cell status. If the cells are easy to fall off the slides then it is recommended to choose to treat with membrane-breaking solution).

v. Immerse and clean the sample in an open beaker containing PBS solution for 2-3 times.

vi. Gently remove excess liquid and carefully drain the liquid around the sample on the slide with filter paper. Keep the sample moist after treatment in a wet box.

d) Cell Smear

i. Resuspend cells in PBS at a concentration of approximately 2×10^7 cells/mL, pipette 50-100 μL of cell suspension onto an anti-defossing slide, and gently spread the cell suspension using a clean slide.

ii. Immerse the slides in a staining jar filled with 4% paraformaldehyde freshly formulated in PBS, fix the cells, and leave at 4 °C for 25 min.

iii. Immerse the slide in PBS, place it at room temperature for 5 min, and repeat once.

iv. Gently remove the excess liquid, and carefully drain the excess liquid around the sample on the slide with filter paper, draw a small circle along the outer contour of the cell with a PAP Pen to facilitate downstream permeability processing and balance labeling operations, and do not allow the sample to dry during the experiment.

v. Immerse samples in the membrane breaking solution and incubate at room temperature for 5 min for permeabilization (Note: It is recommended to use 2-20 μg/mL of Proteinase K working solution for digestion, and treat at 37℃ for about 10 min, depending

on the cell status. If the cells are easy to fall off the slides then it is recommended to choose to treat with membrane-breaking solution).

vi. Immerse and clean the sample in an open beaker containing PBS solution for 2-3 times.

vii. Gently remove excess liquid and carefully drain the liquid around the sample on the slide with filter paper. Keep the sample moist after treatment in a wet box.

2. DNase I Treatment Positive Control Experiment (Optional Steps)

After the sample permeability treatment, treat the sample with DNase I (recommended G3342) to prepare the positive control.

a) Add 100 μL of 1× DNase I buffer (preparation method: mix well 10 μL of 10× DNase I buffer and 90 μL of deionized water) to the permeable sample and incubate at room temperature for 5 min.

b) Gently remove excess liquid and add 100 μL of working solution containing DNase I (20 U/ml), incubate at room temperature for 10 min.

c) Gently remove the excess liquid, and thoroughly wash the slide 3-4 times in the staining tank with PBS;

(**Note**: the positive control slide must use a separate staining cylinder, otherwise the residual DNase I on the positive control slide may introduce a high background on the experimental slide)

3. Marking And Testing

a) Equilibration: Add 50 μL of Equilibration Buffer per sample to cover the sample area and incubate at room temperature for 10 min.

b) Labeling solution preparation: thaw TMR-5-dUTP Labeling Mix and Equilibration Buffer on ice and mix sufficient TdT incubation buffer according to the ratio of Recombinant TdT enzyme: TMR-5-dUTP Labeling Mix: Equilibration Buffer=1 μL: 5 μL: 50 μL (1: 5: 50). The volume of reagents used in specific experiments can be adjusted in an appropriate equal proportion according to the size of the slide.

c) Negative control system: Prepare a control TdT incubation buffer without Recombinant TdT enzyme and replace it with ddH2O.

d) Labeling: remove the equilibration Buffer and then add 56 μL of TdT incubation buffer to each tissue sample and incubate at 37°C for 1 h; Note that the slides should not be dried and protected from light.

e) Immediately wash the tissue samples with PBS 4 times for 5 min each.

f) Gently wipe off the PBS solution around the sample with filter paper.

g) Nuclear staining: Stain the samples in a staining vat and immerse the slides with PI solution or DAPI solution (freshly formulated and diluted with PBS) in the dark at room temperature for 8 min.

h) Sealing: After staining, wash the sample three times with PBS for 5 min each, then gently remove the excess liquid and sealing the slide with the anti-fluorescence quenching sealer (recommended G1401)).

i) Microscopy: Immediately analyze the sample under a fluorescence microscope, the slides are carefully protected from light, PI/DAPI can stain both apoptotic and not apoptotic cells red/blue, red fluorescence localized by TMR-5-dUTP incorporation only in the nuclei of apoptotic cells.

4. Suspension Cells are Detected Using Flow Cytometry

a) The cells to be detected are washed twice with PBS, centrifuged (500 x g) at 4°C and resuspended in 500 μL of PBS.

b) Fixation: Add 5 mL of 1% paraformaldehyde solution prepared with PBS to the sample, fix the cells, and leave on ice for 20 min.

c) Cells are centrifuged at 4°C, 300 x g for 10 min, supernatanted and resuspended twice with 5 mL of PBS, and finally resuspended with 500 μL of PBS.

d) Permeability: Add 5 mL of pre-cooled 70% ethanol on ice to the sample and incubate at -20°C for 4 h to permeabilise the cells.

(**Note**: Cells can also be permeated with membrane breaking solution at room temperature for 5 min)

e) Cells are resuspended with 5 mL of PBS after centrifugation at 300 x g for 10 min, and resuspended with 1 mL of PBS after centrifugation again.

f) Equilibration: Transfer approximately 2×10^6 cells to a 1.5 mL microcentrifuge tube, centrifuge at 300 x g for 10 min, discard the supernatant and resuspend with 80 μL of Equilibration Buffer, incubate at room temperature for 5 min.

g) Labeling solution preparation: thaw TMR-5-dUTP Labeling Mix and Equilibration Buffer on ice and mix sufficient TdT incubation buffer according to the ratio of Recombinant TdT enzyme: TMR-5-dUTP Labeling Mix: Equilibration Buffer=1 μL: 5 μL: 50 μL (1: 5: 50) for all experiments and optional positive control reactions.

h) Labeling: Cells are centrifuged at 300 x g for 10 min, discard the supernatant and resuspend the precipitate in 56 μL of TdT incubation buffer, incubate at 37°C for 1 h, protect from light. Gently resuspend cells with a micropipette every 15 min.

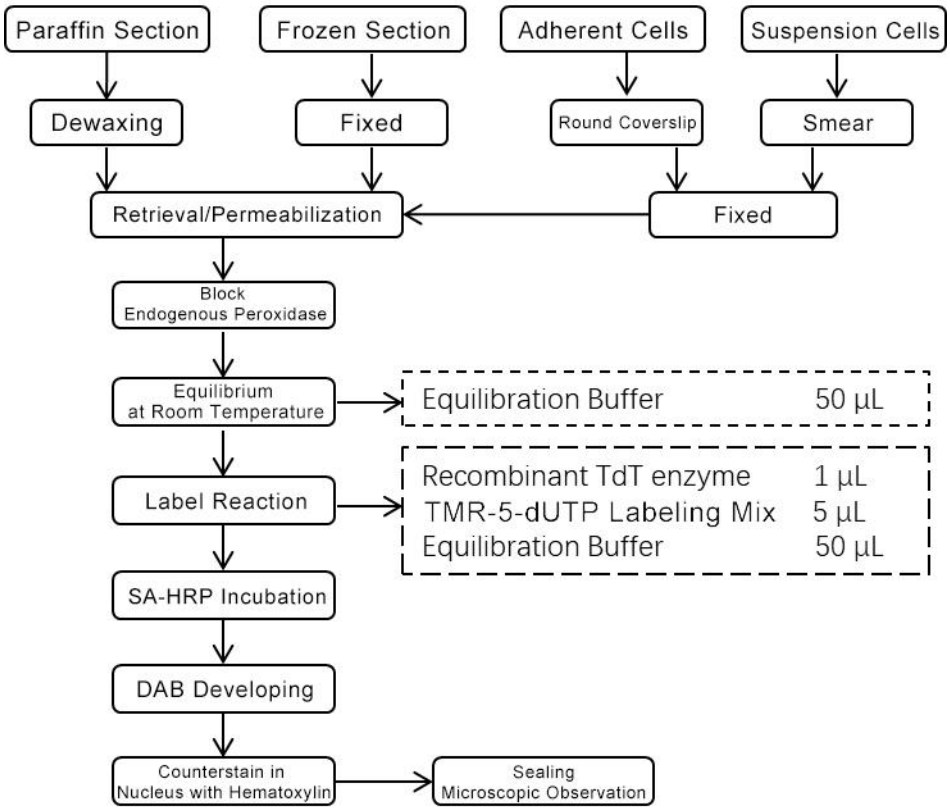
i) After the reaction is complete, add 1 mL of 20 mM EDTA to terminate the reaction and mix gently with a micropipette.

j) Centrifuge at 300 x g for 10 min, discard the supernatant and resuspend the precipitate in 1 mL of membrane breaking solution containing 5 mg/mL BSA, repeat wash twice.

k) Nuclear staining: Centrifuge at 300 x g for 10 min, discard the supernatant and resuspend the cell precipitate in 0.5 mL of PI solution containing 250 μg of RNase A without DNAase, incubate the cells in the dark at room temperature for 30 min.

l) Hands-on detection: Flow cytometry analyzes cells, PI can dye both apoptotic and unpoptoptotic cells blue, and only in the apoptosis nucleus does TMR-5-dUTP incorporate and locate the red fluorescence.

5. Experimental Process Diagram



Note

For your safety and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® CF488 Tunel Cell Apoptosis Detection Kit

Cat #: G1504

Product Information

Product Name	Cat. No	Spec.
CF488 Tunel Cell Apoptosis Detection Kit	G1504-50T	50 T
	G1504-100T	100 T

Product Description/Introduction

The breakage of chromosomal DNA in apoptosis is a gradual process. Chromosomal DNA is first degraded into large fragments of 50-300 KB by endogenous nuclease hydrolase, and then about 30% of chromosomal DNA is randomly cut between nucleosome units under the action of Ca^{2+} and Mg^{2+} dependent nucleic acid endonuclease to form 180-200 bp nucleosome DNA polymers. Therefore, in late apoptosis, DNA is degraded into 180-200 bp fragments, and a large number of 3'-OH terminals are exposed on the broken genomic DNA. Terminal deoxyribonucleotidyl transferase (TDT) is a template independent DNA polymerase, which can catalyze the binding of deoxynucleotides to the 3'-OH terminal of broken DNA molecules. Therefore, TUNEL (TDT mediated dUTP nick end labeling) cell apoptosis detection kit can be used to detect the breakage of nuclear DNA in tissue cells during the late apoptosis. It is based on the incorporation of fluorescein-labelled dUTP (CF488-dUTP) at the exposed 3´-OH ends of genomic DNA breaks by TdT enzymes, which can be detected by fluorescence microscopy or flow cytometry (CF488 excitation 490 nm, emission 515 nm). This kit is suitable for apoptosis detection of paraffin tissue sections, frozen tissue sections, cell crawling, cell smears.

Storage and Shipping Conditions

Ship with wet ice; This kit store at -20°C, CF488-dUTP Labeling Mix store at -20°C, protect from light and valid for 12 months.

Product Components

Component Number	Component	G1504-50T	G1504-100T
G1504-1	Recombinant TdT Enzyme	50 μL	2×50 μL
G1504-2	CF488-dUTP Labeling Mix	250 μL	2×250 μL
G1504-3	Equilibration Buffer	5×1 mL	10×1 mL
G1504-4	Proteinase K (200 μg/mL)	1 mL	2×1 mL
Manual		1 pc	

Product Preparation

1. PBS phosphate buffer (recommended G0002 or G4202).
2. Fixative solution: 4% paraformaldehyde dissolved in PBS, pH 7.4 (recommended G1101).
3. Membrane breaking liquid: 0.1%-0.5% Triton X-100 (recommended G1204).

4. If you need to dye the nucleus, you need to bring your own DAPI (2 μg/mL) or PI (1 μg/mL) (recommended G1012、G1021).
5. For positive control experiments, DNase I is required (recommended G3342).
6. If using a flow cytometer, bring your own PI staining solution (recommended G1021) and RNase A (DNase free) (recommended G3405).
7. For your safety and health, please wear safety glasses, gloves, or protective clothing.

Product Protocol/Procedures

1. Sample Preparation

a) Paraffin Tissue Sections

i. The paraffin tissue sections were immersed in Eco-friendly dewaxing clear solution (G1128) for 5-10 min at room temperature and repeated 3 times; Finally in gradient ethanol (85%, 75%, and double distilled water) once for 5 min each time.

ii. Wash slices gently with PBS and remove the excess liquid around the sample. Use a PAP Pen to draw a small circle 2-3 mm apart along the peripheral contour of the tissue to facilitate downstream permeability treatment and balance labelling. During the experiment, do not let the sample dry, and put the treated sample in the wet box to keep the sample moist.

iii. Prepare Proteinase K working solution: dilute Proteinase K (200 μg/ml) stock solution with PBS at a ratio of 1:9 to a final concentration of 20 μg/mL.

iv. Add 100 μL of Proteinase K working solution to each sample, completely cover the tissue and incubate at 37℃ for 20 min.

Note: Proteinase K contributes to the permeation of staining reagents in the subsequent steps of the tissue and cells. Incubate for too long or too short will affect the efficiency of the subsequent labelling. For better results, the incubation time of Proteinase K can be optimized.

v. Soak and clean the sample with PBS solution for 3 times, each time for 5 min (Proteinase K needs to be cleaned, otherwise it will interfere with the subsequent marking reaction). keep the sample moist in the wet box after treatment.

vi. (optional steps) remove the excess liquid from the sample, add an appropriate amount of membrane breaking liquid to the tissue, fully infiltrate the tissue, and treat at room temperature for 20 min; After the membrane breaking treatment is completed, wash the sample three times with PBS solution for 5 min each time; keep the sample moist after treatment in a wet box.

b) Frozen Tissue Sections

i. Immerse the slides in 4% paraformaldehyde solution (dissolved in PBS) for fixation, and incubate at room temperature for 10-15 min.

ii. Remove the film from the fixation solution and let it dry naturally in a fume hood.

iii. Wash the slide in pure water or PBS to remove residual fixation solution from the slide.

iv. Use a PAP Pen to draw a small circle 2-3 mm apart along the peripheral contour of the tissue to facilitate downstream permeability treatment and balance labelling. During the experiment, do not let the sample dry, and put the treated sample in the wet box to keep the sample moist.

v. Prepare Proteinase K working solution: dilute Proteinase K (200 μg/ml) stock solution with PBS at a ratio of 1:9 to a final concentration of 20 μg/mL.

vi. Add 100 μL of Proteinase K working solution to each sample, completely cover the tissue and incubate at 37℃ for 10 min.

Note: Proteinase K contributes to the permeation of staining reagents in the subsequent steps of the tissue and cells. Incubate for too long or too short will affect the efficiency of the subsequent labelling. For better results, the incubation time of Proteinase K can be optimized.

vii. Rinse the sample with PBS solution for 2-3 times to remove the excess liquid (Proteinase K needs to be cleaned, otherwise it will interfere with the subsequent marking reaction). keep the sample moist in the wet box after treatment.

viii. (optional steps) add an appropriate amount of membrane breaking liquid to the tissue, fully infiltrate the tissue, and treat it at room temperature for 20 min. After completion of the membrane breaking treatment, wash the sample with PBS solution to remove the excess liquid. keep the sample moist after treatment in a wet box.

c) Cell Crawling

i. Adherent cells are cultured on lab-Tek (Chamber Slides). After apoptosis induction treatment, the slides are gently rinsed twice with PBS.

ii. Add an appropriate amount of 4% paraformaldehyde solution (dissolved in PBS) to each slide chamber for fixation, and incubate at room temperature for 20 min.

iii. Remove the fixation solution and wash three times with PBS, each time for 5 min.

iv. Immerse samples in the membrane breaking solution and incubate at room temperature for 5 min for permeabilization (Note: It is recommended to use 2-20 μg/mL of Proteinase K working solution for digestion, and treat at 37℃ for about 10 min, depending on the cell status. If the cells are easy to fall off, it is recommended to use the membrane-breaking solution for treatment.).

v. Immerse and clean the sample in an open beaker containing PBS solution for 2-3 times.

vi. Gently remove excess liquid and carefully drain the liquid around the sample on the slide with filter paper. Keep the sample moist after treatment in a wet box.

d) Cell Smear

i. Resuspend cells in PBS at a concentration of approximately 2×107 cells/mL, pipette 50-100 μL of cell suspension onto an anti-defossing slide, and gently spread the cell suspension using a clean slide.

ii. Immerse the slides in a staining jar filled with 4% paraformaldehyde freshly formulated in PBS, fix the cells, and leave at 4°C for 25 min.

iii. Immerse the slide in PBS, place it at room temperature for 5 min, and repeat once.

iv. Gently remove the excess liquid, and carefully drain the excess liquid around the sample on the slide with filter paper, draw a small circle along the outer contour of the cell with a PAP Pen to facilitate downstream permeability processing and balance labeling operations, and do not allow the sample to dry during the experiment.

v. Immerse samples in the membrane breaking solution and incubate at room temperature for 5 min for permeabilization (Note: It is recommended to use 2-20 μg/mL of Proteinase K working solution for digestion, and treat at 37℃ for about 10 min, depending on the cell status. If the cells are easy to fall off, it is recommended to use the membrane-breaking solution for treatment.).

vi. Immerse and clean the sample in an open beaker containing PBS solution for 2-3 times.

vii. Gently remove excess liquid and carefully drain the liquid around the sample on the slide with filter paper. Keep the sample moist after treatment in a wet box.

2. Dnase I Treatment Positive Control Experiment (Optional Steps)

After the sample permeability treatment, treat the sample with DNase I (recommended G3342) to prepare the positive control.

a) Add 100 μL of 1× DNase I buffer (preparation method: mix well 10 μL of 10× DNase I buffer and 90 μL of deionized water) to the permeable sample and incubate at room temperature for 5 min.

b) Gently remove excess liquid and add 100 μL of working solution containing DNase I (20 U/ml) (preparation method:mix well 10 μL of 10× DNase I buffer, 2 μL of DNase I and 88 μL of deionized water), incubate at room temperature for 10 min.

c) Gently remove the excess liquid, and thoroughly wash the slide 3-4 times in the staining tank with PBS.

(Note: the positive control slide must use a separate staining cylinder, otherwise the residual DNase I on the positive control slide may introduce a high background on the experimental slide).

3. Marking and Testing

d) Equilibration: Add 50 μL of Equilibration Buffer per sample to cover the sample area and incubate at room temperature for 10 min .

e) Labeling solution preparation: thaw CF488-dUTP Labeling Mix and Equilibration Buffer on ice and mix sufficient TdT incubation buffer according to the ratio of Recombinant TdT enzyme:CF488-dUTP Labeling Mix:Equilibration Buffer=1 μL:5 μL:50 μL (1:5:50). The volume of reagents used in specific experiments can be adjusted in an appropriate equal proportion according to the size of the slide.

f) Negative control system: Prepare a control TdT incubation buffer without Recombinant TdT enzyme and replace it with ddH2O.

g) Labeling: remove the equilibration Buffer and then add 56 μL of TdT incubation buffer to each tissue sample and incubate at 37°C for 1 h; Note that the slides should not be dried and protected from light.

h) Immediately wash the tissue samples with PBS four times for 5 min each.

i) Gently wipe off the PBS solution around the sample with filter paper.

j) Nuclear staining: Stain the samples in a staining vat and immerse the slides with PI solution or DAPI solution (freshly formulated and diluted with PBS) in the dark at room temperature for 8 min.

k) Sealing: After staining, wash the sample three times with PBS for 5 min each, then gently remove the excess liquid and sealing the slide with the anti-fluorescence quenching sealer (recommended G1401).

l) Microscopy: Immediately analyze the sample under a fluorescence microscope, the slides are carefully protected from light, PI/DAPI can stain both apoptotic and not apoptotic cells blue, green fluorescence localized by CF488-dUTP incorporation only in the nuclei of apoptotic cells.

4. Suspension Cells Are Detected Using Flow Cytometry

a) The cells to be detected are washed twice with PBS, centrifuged (500 x g) at 4°C and resuspended in 500 μL of PBS.

b) Fixation: Add 5 mL of 1% paraformaldehyde solution prepared with PBS to the sample, fix the cells, and leave on ice for 20 min.

c) Cells are centrifuged at 4°C, 300 x g for 10 min, supernatanted and resuspended twice with 5 mL of PBS, and finally resuspended with 500 μL of PBS.

d) Permeability: Add 5 mL of pre-cooled 70% ethanol on ice to the sample and incubate at -20°C for 4 h to permeabilise the cells.
(Note: Cells can also be permeated with membrane breaking solution at room temperature for 5 min)

e) Cells are resuspended with 5 mL of PBS after centrifugation at 300 x g for 10 min, and resuspended with 1 mL of PBS after centrifugation again.

f) Equilibration: Transfer approximately 2×106 cells to a 1.5 mL microcentrifuge tube, centrifuge at 300 x g for 10 min, discard the supernatant and resuspend with 80 μL of Equilibration Buffer, incubate at room temperature for 5 min.

g) Labeling solution preparation: thaw CF488-dUTP Labeling Mix and Equilibration Buffer on ice and mix sufficient TdT incubation buffer according to the ratio of Recombinant TdT enzyme:CF488-dUTP Labeling Mix:Equilibration Buffer=1 μL:5 μL:50 μL (1 : 5 : 50) for all experiments and optional positive control reactions.

h) Labeling: Cells are centrifuged at 300 x g for 10 min, discard the supernatant and resuspend the precipitate in 56 μL of TdT incubation buffer, incubate at 37°C for 1 h, protect from light. Gently resuspend cells with a micropipette every 15 min.

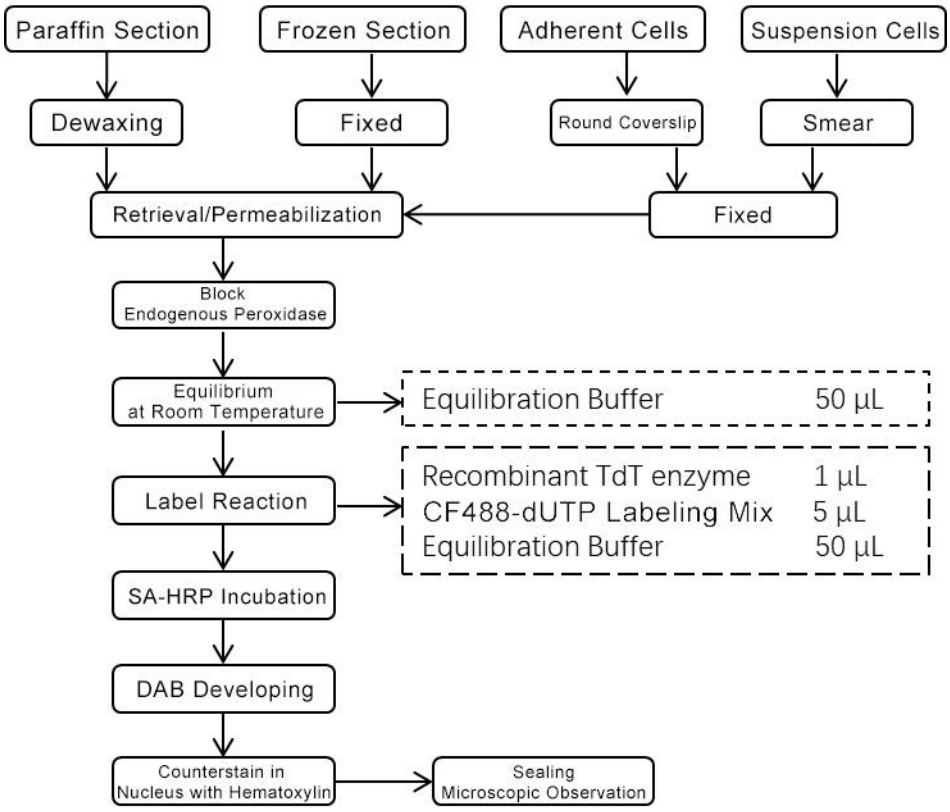
i) After the reaction is complete, add 1 mL of 20 mM EDTA to terminate the reaction and mix gently with a micropipette.

j) Centrifuge at 300 x g for 10 min, discard the supernatant and resuspend the precipitate in 1 mL of membrane breaking solution containing 5 mg/mL BSA, repeat wash twice.

k) Nuclear staining: Centrifuge at 300 x g for 10 min, discard the supernatant and resuspend the cell precipitate in 0.5 mL of PI solution containing 250 μg of RNase A without DNAase, incubate the cells in the dark at room temperature for 30 min.

l) Hands-on detection: Flow cytometry analyzes cells, PI can dye both apoptotic and unpoptoptotic cells blue, and only in the apoptosis nucleus does CF488-dUTP incorporate and locate the green fluorescence.

5. Experimental Process Diagram



Note

For your safety and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® CF488 Tunel Cell Apoptosis Detection Kit

Cat #: G1504

Product Information

Product Name	Cat. No	Spec.
CF488 Tunel Cell Apoptosis Detection Kit	G1504-50T	50 T
	G1504-100T	100 T

Product Description/Introduction

The breakage of chromosomal DNA in apoptosis is a gradual process. Chromosomal DNA is first degraded into large fragments of 50-300 KB by endogenous nuclease hydrolase, and then about 30% of chromosomal DNA is randomly cut between nucleosome units under the action of Ca^{2+} and Mg^{2+} dependent nucleic acid endonuclease to form 180-200 bp nucleosome DNA polymers. Therefore, in late apoptosis, DNA is degraded into 180-200 bp fragments, and a large number of 3'-OH terminals are exposed on the broken genomic DNA. Terminal deoxyribonucleotidyl transferase (TDT) is a template independent DNA polymerase, which can catalyze the binding of deoxynucleotides to the 3'-OH terminal of broken DNA molecules. Therefore, TUNEL (TDT mediated dUTP nick end labeling) cell apoptosis detection kit can be used to detect the breakage of nuclear DNA in tissue cells during the late apoptosis. It is based on the incorporation of fluorescein-labelled dUTP (CF488-dUTP) at the exposed 3´-OH ends of genomic DNA breaks by TdT enzymes, which can be detected by fluorescence microscopy or flow cytometry (CF488 excitation 490 nm, emission 515 nm). This kit is suitable for apoptosis detection of paraffin tissue sections, frozen tissue sections, cell crawling, cell smears.

Storage and Shipping Conditions

Ship with wet ice; This kit store at -20°C, CF488-dUTP Labeling Mix store at -20°C, protect from light and valid for 12 months.

Product Components

Component Number	Component	G1504-50T	G1504-100T
G1504-1	Recombinant TdT Enzyme	50 μL	2×50 μL
G1504-2	CF488-dUTP Labeling Mix	250 μL	2×250 μL
G1504-3	Equilibration Buffer	5×1 mL	10×1 mL
G1504-4	Proteinase K (200 μg/mL)	1 mL	2×1 mL
Manual		1 pc	

Product Preparation

1. PBS phosphate buffer (recommended G0002 or G4202).
2. Fixative solution: 4% paraformaldehyde dissolved in PBS, pH 7.4 (recommended G1101).
3. Membrane breaking liquid: 0.1%-0.5% Triton X-100 (recommended G1204).

4. If you need to dye the nucleus, you need to bring your own DAPI (2 μg/mL) or PI (1 μg/mL) (recommended G1012、G1021).
5. For positive control experiments, DNase I is required (recommended G3342).
6. If using a flow cytometer, bring your own PI staining solution (recommended G1021)and RNase A (DNase free)
7. For your safety and health, please wear safety glasses, gloves, or protective clothing.

Product Protocol/Procedures

1. Sample Preparation

a) Paraffin Tissue Sections

Note: Proteinase K treatment is mainly helpful for the permeability of staining reagents in subsequent steps of tissues and cells. Too long or too short incubation time will affect the subsequent labeling efficiency. In order to obtain better results, the incubation time of Proteinase K can be optimized.

i. Soak paraffin tissue sections in xylene for 5-10 min at room temperature and repeat 2-3 times; Then in anhydrous ethanol for 5 min and repeated twice; Finally in gradient ethanol (85%, 75%, and double distilled water) once for 5 min each time.

ii. Wash slices gently with PBS and remove the excess liquid around the sample. Use a histochemical pen to draw a small circle 2-3 mm apart along the peripheral contour of the tissue to facilitate downstream permeability treatment and balance labelling. During the experiment, do not let the sample dry, and put the treated sample in the wet box to keep the sample moist.

iii. Prepare Proteinase K working solution: dilute Proteinase K (200 μg/ml) stock solution with PBS at a ratio of 1:9 to a final concentration of 20 μg/mL.

iv. Add 100 μL of Proteinase K working solution to each sample, completely cover the tissue and incubate at 37℃ for 20 min.

Note: Proteinase K contributes to the permeation of staining reagents in the subsequent steps of the tissue and cells. Incubate for too long or too short will affect the efficiency of the subsequent labelling. For better results, the incubation time of Proteinase K can be optimized.

v. Soak and clean the sample with PBS solution for 3 times, each time for 5 min (Proteinase K needs to be cleaned, otherwise it will interfere with the subsequent marking reaction). keep the sample moist in the wet box after treatment.

vi. (optional steps) remove the excess liquid from the sample, add an appropriate amount of membrane breaking liquid to the tissue, fully infiltrate the tissue, and treat at room temperature for 20 min; After the membrane breaking treatment is completed, wash the sample three times with PBS solution for 5 min each time; keep the sample moist after treatment in a wet box.

b) Frozen Tissue Sections

i. Immerse the slides in 4% paraformaldehyde solution (dissolved in PBS) for fixation, and incubate at room temperature for 10-15 min.

ii. Remove the film from the fixation solution and let it dry naturally in a fume hood.

iii. Wash the slide in pure water or PBS to remove residual fixation solution from the slide.

iv. Use a histochemical pen to draw a small circle 2-3 mm apart along the peripheral contour of the tissue to facilitate downstream permeability treatment and balance labelling. During the experiment, do not let the sample dry, and put the treated sample in the wet box to keep moist.

v. Prepare Proteinase K working solution: dilute Proteinase K (200 μg/ml) stock solution with PBS at a ratio of 1:9 to a final concentration of 20 μg/mL.

vi. Add 100 μL of Proteinase K working solution to each sample, completely cover the tissue and incubate at 37℃ for 10 min.

Note: Proteinase K contributes to the permeation of staining reagents in the subsequent steps of the tissue and cells. Incubate for too long or too short will affect the efficiency of the subsequent labelling. For better results, the incubation time of Proteinase K can be optimized.

vii. Rinse the sample with PBS solution for 2-3 times to remove the excess liquid (Proteinase K needs to be cleaned, otherwise it will interfere with the subsequent marking reaction). keep the sample moist in the wet box after treatment.

viii. (optional steps) add an appropriate amount of membrane breaking liquid to the tissue, fully infiltrate the tissue, and treat it at room temperature for 20 min. After completion of the membrane breaking treatment, wash the sample with PBS solution to remove the excess liquid. keep the sample moist after treatment in a wet box.

c) Cell Crawling

i. Adherent cells are cultured on lab-Tek (Chamber Slides). After apoptosis induction treatment, the slides are gently rinsed twice with PBS.

ii. Add an appropriate amount of 4% paraformaldehyde solution (dissolved in PBS) to each slide chamber for fixation, and incubate at room temperature for 20 min.

iii. Remove the fixation solution and wash three times with PBS, each time for 5 min.

iv. Immerse samples in the membrane breaking solution and incubate at room temperature for 5 min for permeabilisation.

v. Immerse and clean the sample in an open beaker containing PBS solution for 2-3 times.

vi. Gently remove excess liquid and carefully drain the liquid around the sample on the slide with filter paper. Keep the sample moist after treatment in a wet box.

d) Cell Smear

i. Resuspend cells in PBS at a concentration of approximately 2×10^7 cells/mL, pipette 50-100 μL of cell suspension onto an anti-defossing slide, and gently spread the cell suspension using a clean slide.

ii. Immerse the slides in a staining jar filled with 4% paraformaldehyde freshly formulated in PBS, fix the cells, and leave at 4°C for 25 min.

iii. Immerse the slide in PBS, place it at room temperature for 5 min, and repeat once.

iv. Gently remove the excess liquid, and carefully drain the excess liquid around the sample on the slide with filter paper, draw a small circle along the outer contour of the cell with a histochemical pen to facilitate downstream permeability processing and balance labeling operations, and do not allow the sample to dry during the experiment.

v. Immerse samples in the membrane breaking solution and incubate at room temperature for 5 min for permeabilisation.

vi. Immerse and clean the sample in an open beaker containing PBS solution for 2-3 times.

vii. Gently remove excess liquid and carefully drain the liquid around the sample on the slide with filter paper. Keep the sample moist after treatment in a wet box.

2. Dnase I Treatment Positive Control Experiment (Optional Steps)

After the sample permeability treatment, treat the sample with DNase I (recommended G3342) to prepare the positive control.

a) Add 100 μL of 1× DNase I buffer (preparation method: mix well 10 μL of 10× DNase I buffer and 90 μL of deionized water) to the permeable sample and incubate at room temperature for 5 min.

b) Gently remove excess liquid and add 100 μL of working solution containing DNase I (20 U/ml) (preparation method:mix well 10 μL of 10× DNase I buffer, 2 μL of DNase I and 88 μL of deionized water), incubate at room temperature for 10 min.

c) Gently remove the excess liquid, and thoroughly wash the slide 3-4 times in the staining tank with PBS.

(**Note**: the positive control slide must use a separate staining cylinder, otherwise the residual DNase I on the positive control slide may introduce a high background on the experimental slide).

3. Marking and Testing

a) Equilibration: Add 50 μL of Equilibration Buffer per sample to cover the sample area and incubate at room temperature for 10 min .

b) Labeling solution preparation: thaw CF488-dUTP Labeling Mix and Equilibration Buffer on ice and mix sufficient TdT incubation buffer according to the ratio of Recombinant TdT enzyme:CF488-dUTP Labeling Mix:Equilibration Buffer=1 μL:5 μL:50 μL (1:5:50). The volume of reagents used in specific experiments can be adjusted in an appropriate equal proportion according to the size of the slide.

c) Negative control system: Prepare a control TdT incubation buffer without Recombinant TdT enzyme and replace it with ddH_2O.

d) Labeling: remove the equilibration Buffer and then add 56 μL of TdT incubation buffer to each tissue sample and incubate at 37°C for 1 h; Note that the slides should not be dried and protected from light.

e) Immediately wash the tissue samples with PBS four times for 5 min each.

f) Gently wipe off the PBS solution around the sample with filter paper.

g) Nuclear staining: Stain the samples in a staining vat and immerse the slides with PI solution or DAPI solution (freshly formulated and diluted with PBS) in the dark at room temperature for 8 min.

h) Sealing: After staining, wash the sample three times with PBS for 5 min each, then gently remove the excess liquid and sealing the slide with the anti-fluorescence quenching sealer (recommended G1401)).

i) Microscopy: Immediately analyze the sample under a fluorescence microscope, the slides are carefully protected from light, PI/DAPI can stain both apoptotic and not apoptotic cells blue, green fluorescence localized by CF488-dUTP incorporation only in the nuclei of apoptotic cells.

4. Suspension Cells Are Detected Using Flow Cytometry

a) The cells to be detected are washed twice with PBS, centrifuged (500 x g) at 4°C and resuspended in 500 μL of PBS.

b) Fixation: Add 5 mL of 1% paraformaldehyde solution prepared with PBS to the sample, fix the cells, and leave on ice for 20 min.

c) Cells are centrifuged at 4°C, 300 x g for 10 min, supernatanted and resuspended twice with 5 mL of PBS, and finally resuspended with 500 μL of PBS.

d) Permeability: Add 5 mL of pre-cooled 70% ethanol on ice to the sample and incubate at -20°C for 4 h to permeabilise the cells.

(**Note**: Cells can also be permeated with membrane breaking solution at room temperature for 5 min)

e) Cells are resuspended with 5 mL of PBS after centrifugation at 300 x g for 10 min, and resuspended with 1 mL of PBS after centrifugation again.

f) Equilibration: Transfer approximately 2×10^6 cells to a 1.5 mL microcentrifuge tube, centrifuge at 300 x g for 10 min, discard the supernatant and resuspend with 80 μL of Equilibration Buffer, incubate at room temperature for 5 min.

g) Labeling solution preparation: thaw CF488-dUTP Labeling Mix and Equilibration Buffer on ice and mix sufficient TdT incubation buffer according to the ratio of Recombinant TdT enzyme:CF488-dUTP Labeling Mix:Equilibration Buffer=1 μL:5 μL:50 μL (1 : 5 : 50) for all experiments and optional positive control reactions.

h) Labeling: Cells are centrifuged at 300 x g for 10 min, discard the supernatant and resuspend the precipitate in 56 μL of TdT incubation buffer, incubate at 37°C for 1 h, protect from light. Gently resuspend cells with a micropipette every 15 min.

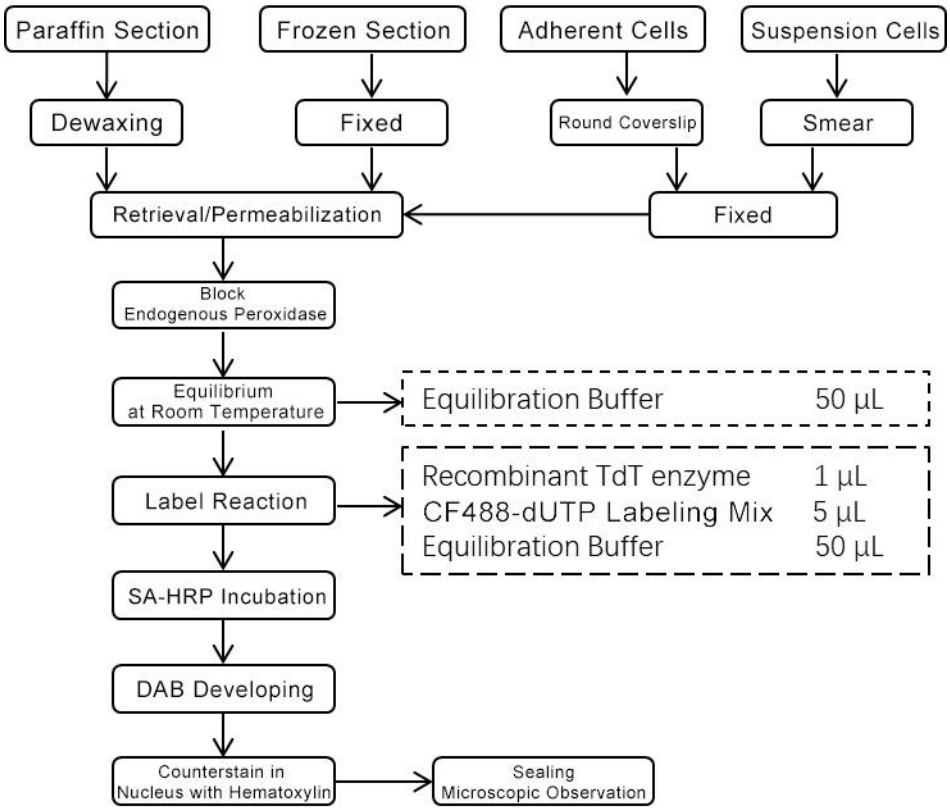
i) After the reaction is complete, add 1 mL of 20 mM EDTA to terminate the reaction and mix gently with a micropipette.

j) Centrifuge at 300 x g for 10 min, discard the supernatant and resuspend the precipitate in 1 mL of membrane breaking solution containing 5 mg/mL BSA, repeat wash twice.

k) Nuclear staining: Centrifuge at 300 x g for 10 min, discard the supernatant and resuspend the cell precipitate in 0.5 mL of PI solution containing 250 μg of RNase A without DNAase, incubate the cells in the dark at room temperature for 30 min.

l) Hands-on detection: Flow cytometry analyzes cells, PI can dye both apoptotic and unpoptoptotic cells blue, and only in the apoptosis nucleus does CF488-dUTP incorporate and locate the green fluorescence.

5. Experimental Process Diagram



Note

For your safety and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® CF640 Tunel Cell Apoptosis Detection Kit

Cat #: G1505

Product Information

Product Name	Cat. No	Spec.
CF640 Tunel Cell Apoptosis Detection Kit	G1505-50T	50 T
	G1505-100T	100 T

Product Description/Introduction

The breakage of chromosomal DNA in apoptosis is a gradual process. Chromosomal DNA is first degraded into large fragments of 50-300 KB by endogenous nuclease hydrolase, and then about 30% of chromosomal DNA is randomly cut between nucleosome units under the action of Ca^{2+} and Mg^{2+} dependent nucleic acid endonuclease to form 180-200 bp nucleosome DNA polymers. Therefore, in late apoptosis, DNA is degraded into 180-200 bp fragments, and a large number of 3'-OH terminals are exposed on the broken genomic DNA. Terminal deoxyribonucleotidyl transferase (TDT) is a template independent DNA polymerase, which can catalyze the binding of deoxynucleotides to the 3'-OH terminal of broken DNA molecules. Therefore, TUNEL (TDT mediated dUTP nick end labeling) cell apoptosis detection kit can be used to detect the breakage of nuclear DNA in tissue cells during the late apoptosis. It is based on the incorporation of fluorescein-labelled dUTP (CF640-dUTP) at the exposed 3´-OH ends of genomic DNA breaks by TdT enzymes, which can be detected by fluorescence microscopy or flow cytometry (CF640 excitation 642 nm, emission 662 nm). This kit has a wide range of applications and is suitable for apoptosis detection in paraffin tissue sections, frozen tissue sections, cell crawls and cell smears.

Storage and Shipping Conditions

Ship with wet ice; This kit store at -20°C, CF640-dUTP Labeling Mix store at -20 °C, protect from light and valid for 12 months.

Product Components

Component Number	Component	G1505-50T	G1505-100T
G1505-1	Recombinant TdT Enzyme	50 μL	2×50 μL
G1505-2	CF640-dUTP Labeling Mix	250 μL	2×250 μL
G1505-3	Equilibration Buffer	5×1 mL	10×1 mL
G1505-4	Proteinase K (200 μg/mL)	1 mL	2×1 mL
Manual		1 pc	

Preparation

1. PBS phosphate buffer (recommended G0002 or G4202).
2. Fixative solution: 4% paraformaldehyde dissolved in PBS, pH 7.4 (recommended G1101).
3. Membrane breaking liquid: 0.1%-0.5% Triton X-100 (recommended G1204).

4. If you need to dye the nucleus, you need to bring your own DAPI (2 μg/mL) or PI (1 μg/mL) (recommended G1012, G1021).
5. For positive control experiments, DNase I is required (recommended G3342).
6. If using a flow cytometer, bring your own PI staining solution (recommended G1021) and RNase A (DNase free) (recommended G3405).
7. For your safety and health, please wear safety glasses, gloves, or protective clothing.

Product Protocol/Procedures

1. Sample Preparation

a) Paraffin Tissue Sections

i. Soak paraffin tissue sections in environmentally friendly dewaxing clear solution (G1128) for 5-10 min at room temperature and repeat 3 times; Then in anhydrous ethanol for 5 min and repeated twice; Finally in gradient ethanol (85%, 75%, and double distilled water) once for 5 min each time.

ii. Wash slices gently with PBS and remove the excess liquid around the sample. Use a PAP Pen to draw a small circle 2-3 mm apart along the peripheral contour of the tissue to facilitate downstream permeability treatment and balance labelling. During the experiment, do not let the sample dry, and put the treated sample in the wet box to keep the sample moist.

iii. Prepare Proteinase K working solution: dilute Proteinase K (200 μg/ml) stock solution with PBS at a ratio of 1:9 to a final concentration of 20 μg/mL.

iv. Add 100 μL of Proteinase K working solution to each sample, completely cover the tissue and incubate at 37℃ for 20 min.

Note: Proteinase K contributes to the permeation of staining reagents in the subsequent steps of the tissue and cells. Incubate for too long or too short will affect the efficiency of the subsequent labelling. For better results, the incubation time of Proteinase K can be optimized.

v. Soak and clean the sample with PBS solution for 3 times, each time for 5 min (Proteinase K needs to be cleaned, otherwise it will interfere with the subsequent marking reaction). Keep the sample moist in the wet box after treatment.

vi. (optional steps) remove the excess liquid from the sample, add an appropriate amount of membrane breaking liquid to the tissue, fully infiltrate the tissue, and treat at room temperature for 20 min; After the membrane breaking treatment is completed, wash the sample three times with PBS solution for 5 min each time; keep the sample moist after treatment in a wet box.

b) Frozen Tissue Sections

i. Immerse the slides in 4% paraformaldehyde solution (dissolved in PBS) for fixation, and incubate at room temperature for 10-15 min.

ii. Remove the film from the fixation solution and let it dry naturally in a fume hood.

iii. Wash the slide in pure water or PBS to remove residual fixation solution from the slide.

iv. Use a PAP Pen to draw a small circle 2-3 mm apart along the peripheral contour of the tissue to facilitate downstream permeability treatment and balance labelling. During the experiment, do not let the sample dry, and put the treated sample in the wet box to keep moist.

v. Prepare Proteinase K working solution: dilute Proteinase K (200 μg/ml) stock solution with PBS at a ratio of 1:9 to a final concentration of 20 μg/mL.

vi. Add 100 μL of Proteinase K working solution to each sample, completely cover the tissue and incubate at 37℃ for 10 min.

Note: Proteinase K contributes to the permeation of staining reagents in the subsequent steps of the tissue and cells. Incubate for too long or too short will affect the efficiency of the subsequent labelling. For better results, the incubation time of Proteinase K can be optimized.

vii. Rinse the sample with PBS solution for 2-3 times to remove the excess liquid (Proteinase K needs to be cleaned, otherwise it will interfere with the subsequent marking reaction). keep the sample moist in the wet box after treatment.

viii. (optional steps) add an appropriate amount of membrane breaking liquid to the tissue, fully infiltrate the tissue, and treat it at room temperature for 20 min. After completion of the membrane breaking treatment, wash the sample with PBS solution to remove the excess liquid. keep the sample moist after treatment in a wet box.

c) Cell Crawling

i. Adherent cells are cultured on lab-Tek (Chamber Slides). After apoptosis induction treatment, the slides are gently rinsed twice with PBS.

ii. Add an appropriate amount of 4% paraformaldehyde solution (dissolved in PBS) to each slide chamber for fixation, and incubate at room temperature for 20 min.

iii. Remove the fixation solution and wash three times with PBS, each time for 5 min.

iv. Immerse samples in the membrane breaking solution and incubate at room temperature for 5 min for permeabilization (Note: It is recommended to use 2-20 μg/mL of Proteinase K working solution for digestion, and treat at 37℃ for about 10 min, depending on the cell status. If the cells are prone to drop off, it is recommended to treat them with membrane-breaking solution)

v. Wash the samples by submerging them 2-3 times in an open beaker with PBS solution.

vi. Gently remove excess liquid and carefully drain the liquid around the sample on the slide with filter paper. Keep the sample moist after treatment in a wet box.

d) Cell Smear

i. Resuspend cells in PBS at a concentration of approximately 2×107 cells/mL, pipette 50-100 μL of cell suspension onto an anti-defossing slide, and gently spread the cell suspension using a clean slide.

ii. Immerse the slides in a staining jar filled with 4% paraformaldehyde freshly formulated in PBS, fix the cells, and leave at 4°C for 25 min.

iii. Immerse the slide in PBS, place it at room temperature for 5 min, and repeat once.

iv. Gently remove the excess liquid, and carefully drain the excess liquid around the sample on the slide with filter paper, draw a small circle along the outer contour of the cell with a PAP Pen to facilitate downstream permeability processing and balance labeling operations, and do not allow the sample to dry during the experiment.

v. Immerse samples in the membrane breaking solution and incubate at room temperature for 5 min for permeabilization (Note: It is recommended to use 2-20 μg/mL of Proteinase K working solution for digestion, and treat at 37℃ for about 10 min, depending on the cell

status. If the cells are prone to drop off, it is recommended to treat them with membrane-breaking solution)

vi. Wash the samples by submerging them 2-3 times in an open beaker with PBS solution.

vii. Gently remove excess liquid and carefully drain the liquid around the sample on the slide with filter paper. Keep the sample moist after treatment in a wet box.

2. Dnase I Treatment Positive Control Experiment (Optional Steps)

After the sample permeability treatment, treat the sample with DNase I (recommended G3342) to prepare the positive control.

a) Add 100 μL of 1× DNase I buffer (preparation method: mix well 10 μL of 10× DNase I buffer and 90 μL of deionized water) to the permeable sample and incubate at room temperature for 5 min.

b) Gently remove excess liquid and add 100 μL of working solution containing DNase I (20 U/ml) (preparation method: mix well 10 μL of 10× DNase I buffer, 2 μL of DNase I and 88 μL of deionized water), incubate at room temperature for 10 min.

c) Gently remove the excess liquid, and thoroughly wash the slide 3-4 times in the staining tank with PBS.

(Note: the positive control slide must use a separate staining cylinder, otherwise the residual DNase I on the positive control slide may introduce a high background on the experimental slide).

3. Marking and Testing

d) Equilibration: Add 50 μL of Equilibration Buffer per sample to cover the sample area and incubate at room temperature for 10 min.

e) Labeling solution preparation: Thaw CF640-5-dUTP Labeling Mix and Equilibration Buffer on ice and mix sufficient TdT incubation buffer according to the ratio of Recombinant TdT enzyme:CF640-5-dUTP Labeling Mix:Equilibration Buffer=1 μL:5 μL:50 μL (1:5:50) ratio. The volume of reagents used in specific experiments can be adjusted in an appropriate equal proportion according to the size of the slide.

f) Negative control system: Prepare a control TdT incubation buffer without Recombinant TdT enzyme and replace it with ddH2O.

g) Labeling: remove the equilibration Buffer and then add 56 μL of TdT incubation buffer to each tissue sample and incubate at 37°C for 1 h; Note that the slides should not be dried and protected from light.

h) Immediately wash the tissue samples with PBS and wash 4 times for 5 min each.

i) Gently wipe off the PBS solution around the sample with filter paper.

j) Nuclear staining: Stain the samples in a staining vat and immerse the slides with PI solution or DAPI solution (freshly formulated and diluted with PBS) in the dark at room temperature for 8 min.

k) Sealing: After staining, wash the sample three times with PBS for 5 min each, then gently remove the excess liquid and sealing the slide with the anti-fluorescence quenching sealer (recommended G1401)).

l) Microscopy: Immediately analyze the sample under a fluorescence microscope, the slides are carefully protected from light, PI/DAPI can stain both apoptotic and not apoptotic cells blue, pink fluorescence localized by CF640-5-dUTP incorporation only in the nuclei of apoptotic cells.

4. Suspension Cells are Detected Using Flow Cytometry

a) The cells to be detected are washed twice with PBS, centrifuge (500 x g) at 4°C and resuspended in 500 μL of PBS.

b) Fixation: Add 5 mL of 1% paraformaldehyde solution prepared with PBS to the sample, fix the cells, and leave on ice for 20 min.

c) Cells are centrifuged at 4°C, 300 x g for 10 min, supernatanted and resuspended twice with 5 mL of PBS, and finally resuspended with 500 μL of PBS.

d) Permeability: Add 5 mL of pre-cooled 70% ethanol on ice to the sample and incubate at -20°C for 4 h to permeabilise the cells.
(Note: Cells can also be permeated with membrane breaking solution at room temperature for 5 min)

e) Cells are resuspended with 5 mL of PBS after centrifugation at 300 x g for 10 min, resuspended with 1 mL of PBS after centrifugation again.

f) Equilibration: Transfer approximately 2×106 cells to a 1.5 mL microcentrifuge tube, centrifuge at 300 x g for 10 min, discard the supernatant and resuspend with 80 μL of Equilibration Buffer, incubate at room temperature for 5 min.

g) Labeling solution preparation: Thaw CF640-dUTP Labeling Mix and Equilibration Buffer on ice and mix sufficient TdT incubation buffer according to the ratio of Recombinant TdT enzyme:CF640-dUTP Labeling Mix:Equilibration Buffer=1 μL:5 μL:50 μL (1:5:50) for all experiments and optional positive control reactions.

h) Labeling: Cells are centrifuged at 300 x g for 10 min, discard the supernatant and resuspend the precipitate in 56 μL of TdT incubation buffer, incubate at 37°C for 1 h, protecte from light. Gently resuspend cells with a micropipette every 15 min.

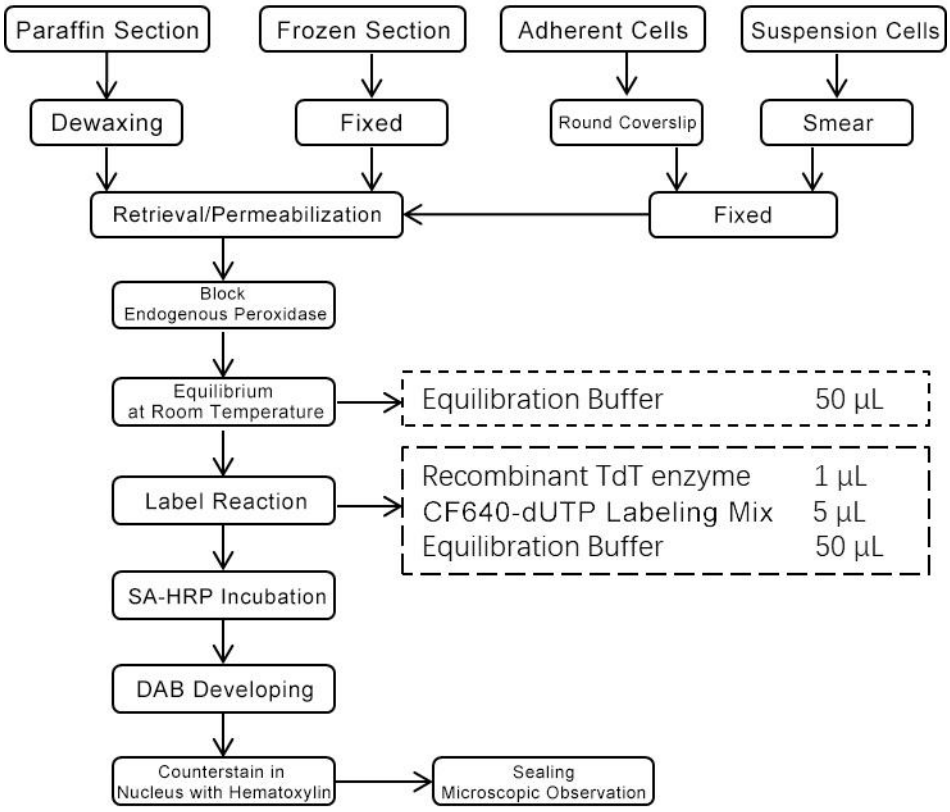
i) After the reaction is complete, add 1 mL of 20 mM EDTA to terminate the reaction and mix gently with a micropipette.

j) Centrifuge at 300 x g for 10 min, discard the supernatant and resuspend the precipitate in 1 mL of membrane breaking solution containing 5 mg/mL BSA, repeat wash twice.

k) Nuclear staining: Centrifuge at 300 x g for 10 min, discard the supernatant and resuspend the cell precipitate in 0.5 mL of PI solution containing 250 μg of RNase A without DNAase, incubate the cells in the dark at room temperature for 30 min.

l) Hands-on detection: Flow cytometry analyzes cells, PI can dye both apoptotic and unpoptoptotic cells blue, and only in the apoptosis nucleus does CF640-dUTP incorporate and locate the pink fluorescence.

5. Experimental Process Diagram



Note

For your safety and health, please wear safety glasses, gloves, or protective clothing.

fServicebio® Click-iT 488 Tunel Cell Apoptosis Detection Kit

Cat. No.: G1506-50T

Product Information

Product Name	Cat. No.	Spec.
Click-iT 488 Tunel Cell Apoptosis Detection Kit	G1506-50T	50T

Product Description/Introduction

The breaking of chromosomal DNA in apoptosis is a gradual, phased process. Chromosomal DNA is first degraded into large 50-300 kb segments by endogenous nucleic acid hydrolases, and then about 30% of chromosomal DNA is randomly cut between nucleosome units in the presence of Ca2+- and Mg2+-dependent nucleic acid endonucleases to form 180-200 bp nucleosomal DNA multimers.Thus, in late apoptosis, DNA is degraded into 180-200 bp fragments, and a large number of 3'-OH ends are exposed on the broken genomic DNA. Terminal Deoxynucleotidyl Transferase (TdT) is a template-independent DNA polymerase that catalyzes the incorporation of deoxyribonucleotides into the 3'-OH ends of broken DNA molecules.Therefore TUNEL (TdT mediated dUTP Nick End Labeling) Cell Apoptosis Detection Kit can be used to detect the breakage of nuclear DNA in tissue cells during late apoptosis.

The principle is that under the action of TdT enzyme, EdUTP (a dUTP with alkyne modification) is doped into the 3´-OH end exposed during genomic DNA breakage, and the alkyne group reacts with the azide dye in a ring-forming reaction catalyzed by a monovalent copper ion (click reaction) thus introducing the fluorescent moiety in a targeted way. iF488 azide is used in this kit, thus it can be detected by fluorescence microscope or flow cytometry (iF488 excitation 491 nm, emission 516 nm). Compared to other modified dUTPs, EdUTP has a smaller spatial site resistance and is more easily doped into DNA ends by TdTase.

This kit has a wide range of applications and is suitable for apoptosis detection in paraffin tissue sections, frozen tissue sections, cell crawls and cell smears.

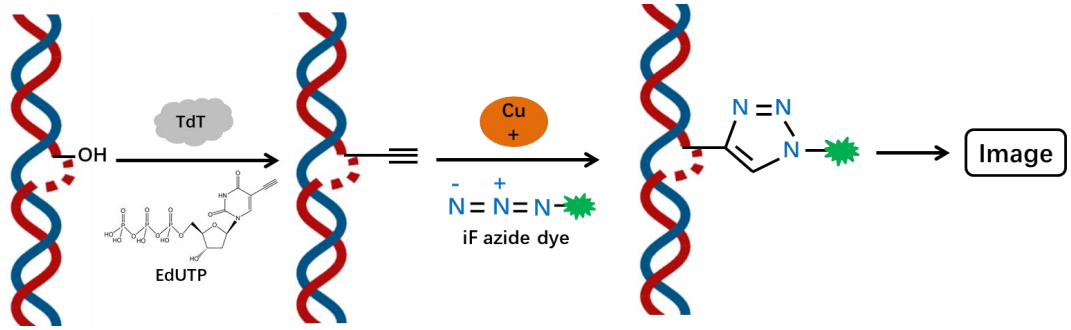


Figure 1. Schematic diagram of the click chemistry-based TUNEL kit principle

Storage and Shipping Conditions

Ship with wet ice; Store at -20℃ for 12 months.

Product Content

Component Number	Component	G1506-50T
G1506-1	Recombinant Tdt Enzyme	50 μL
G1506-2	EdUTP Labeling Mix	250 μL
G1506-3	Equilibration Buffer	5×1 mL

G1506-4	Proteinase K (200μg/mL)	1 mL
G1506-5	iF488 azide dye	80 μL
G1506-6	Reaction Buffer A	5×1 mL
G1506-7	Reaction Buffer B	60 μL
G1506-8	Reaction additive (Reagent C)	2×100 mg
Manual		1 pc

Pre-experimentation

1. PBS phosphate buffer (recommended G0002 or G4202);
2. Fixed solution: 4% paraformaldehyde dissolved in PBS, pH 7.4 (recommended G1101);
3. Membrane-breaking solution: 0.1% Triton X-100 dissolved in 0.1% sodium citrate (recommended G1204);
4. 0.2% Triton X-100 prepared in PBS; 0.1% Triton X-100 prepared in PBS containing 5 mg/mL BSA;
5. For nuclear staining, provide your own DAPI (2 μg/mL), Hoechst 33258, or PI (1 μg/mL) (G1012, G1011, G1021 recommended);
6. DNase I (G3342) for positive control experiments;
7. Reaction additive (Reagent C) is centrifuged at low speed, 100 mg of the powder is dissolved in 1 mL of ultrapure water (ready to use), and 100 μL is dispensed and stored at -20°C, leaving the remaining powder for spare use; (Reagent C is easy to oxidize, try to avoid long time exposure to the air, after formulated into an aqueous solution, it is strongly recommended to divide into small portions for use; After testing the aqueous solution of Reagent C, the color of a slight change, click the reaction system can still be carried out normally, and if it shows brown color, it indicates that the component has been invalidated)
8. If the background color of the result is too dark, it may be caused by insufficient washing and residual fixative during the experiment;
9. Be careful to add the reaction liquid in sequence, and mix well while adding;
10. For your health and safety, please wear lab coat and gloves during operation.

Assay Protocol / Procedures

1. Sample Preparation

A. Paraffin-embedded tissue sections

a. The paraffin tissue sections were immersed in Eco-friendly dewaxing clear solution (G1128) for 5-10 min at room temperature and repeated 3 times. Then soak in anhydrous ethanol for 5 min and repeat twice; Finally, soak in gradient ethanol (85%, 75%, and double-distilled water) once for 5 min each;

b. Gently moisten the section with PBS and remove excess liquid around the sample, use a PAP Pen to draw a small circle spaced 2-3 mm apart from the tissue along the tissue's peripheral contour to facilitate downstream permeability processing and equilibrium labeling operations, do not allow the sample to dry out during experiments, and keep the processed samples in a wet box to keep the samples moist;

c. **Preparation of Proteinase K working solution**: Dilute the Proteinase K (200 μg/mL) stock solution with PBS as diluent at a ratio of 1:9 to give a final concentration of 20 μg/mL;

d. Add 100 μL of the above Proteinase K working solution to each sample to make it fully covered, and incubate at 37°C for 20 min;

Note: Proteinase K treatment mainly contributes to the permeation of staining reagents in the subsequent steps of tissues and cells, and its incubation time is too long or too short to affect the

efficiency of the subsequent labeling, in order to get better results, the incubation time can be optimized according to the actual situation.

e. Wash the samples by moistening with PBS solution 3 times for 5 min each time (Proteinase K needs to be washed clean or it will interfere with the subsequent labeling reaction). The treated samples were placed in a wet box to keep the samples moist;

f. (Optional step) Remove the excess liquid on the sample, add appropriate amount of membrane-breaking solution droplets to the tissue, fully infiltrate the tissue, and treat it for 20 min at room temperature; after the membrane-breaking treatment is completed, the sample is similarly washed with PBS solution for 3 times, each time for 5 min; the treated sample is placed in a wet box to keep the sample moist.

B. Tissue frozen section

a. The tissue frozen sections are submerged in the fixative and incubated and fixed for 10-15 min at room temperature;

b. The tissue sections are removed from the fixative and placed in a fume hood to dry naturally;

c. Tissue sections are moistened and washed in purified water or PBS to remove residual fixative from the sample;

d. Use a PAP Pen to draw a small circle spaced 2-3 mm apart from the tissue along the tissue's peripheral contour to facilitate downstream permeability processing and equilibrium labeling operations, do not allow the sample to dry out during experiments, and keep the processed samples in a wet box to keep the samples moist;

e. Preparation of Proteinase K working solution: Dilute the Proteinase K (200 μg/mL) stock solution with PBS as diluent at a ratio of 1:9 to give a final concentration of 20 μg/mL;

f. Add 100 μL of the above Proteinase K working solution to each sample to make it fully covered, and incubate for 10 min at room temperature;

Note: Proteinase K treatment mainly contributes to the permeation of staining reagents in the subsequent steps of tissues and cells, and its incubation time is too long or too short to affect the efficiency of the subsequent labeling, in order to get better results, the incubation time can be optimized according to the actual situation.

g. Wash the sample 2-3 times with PBS solution to remove excess liquid (Proteinase K needs to be washed clean or it will interfere with the subsequent labeling reaction), and keep the treated sample in a wet box to keep the sample moist;

h. (Optional step) Add appropriate amount of membrane-breaking solution droplets to the tissue, fully infiltrate the tissue, room temperature treatment for 20 min, membrane-breaking treatment is completed the same after the completion of the sample with the PBS solution to rinse, to remove the excess liquid, after the treatment of the sample in the wet box to keep the sample wet.

C. Cell creep

a. Adherent cells are cultured on Lab-Tek slide chambers (Chamber Slides), and slides are gently moistened and washed 2 times with PBS after apoptosis induction treatment;

b. Appropriate amount of fixative is added to each slide chamber to cover the tissue and incubated for 20 min at room temperature;

c. Remove the fixative and add PBS to wash 3 times for 5 min each;

d. Each sample is immersed in 0.2% Triton X-100 solution prepared in PBS and incubated for 5 min at room temperature for permeabilization;

e. Wash samples by submerging them 2-3 times in an open beaker containing PBS solution;

f. Gently remove excess liquid and use filter paper to carefully blot the liquid around the sample on the slide.The processed sample is placed in a wet box to keep the sample moist.

D. Cell smear

a. Cells are resuspended in PBS at a concentration of about 2 × 10^7 cells/mL, and 50-100 μL of cell suspension is aspirated onto an anti-detachment slide, and a clean slide is used to gently spread the cell suspension;

b. The cell smears are immersed in a staining jar filled with fixing solution, and the cells are fixed and left at 4°C for 25 min;

c. Immerse the slide in PBS and leave it at room temperature for 5 min to soak and rinse, repeat once;

d. Gently remove the excess liquid and carefully blot the excess liquid around the sample on the slide with filter paper, draw a small circle along the peripheral contour of the cell with a PAP Pen to facilitate downstream permeability processing and equilibrium labeling operations, and do not allow the sample to dry out during the course of the experiment;

e. Each sample is immersed in 0.2% Triton X-100 solution prepared in PBS and incubated for 5 min at room temperature for permeabilization;

f. Wash samples by submerging them 2-3 times in an open beaker containing PBS solutio

g. Gently remove excess liquid and carefully blot the liquid around the sample on the slide with filter paper. Keep the sample moist by placing the treated sample in a wet box.

2. DNase I treatment positive control experiment (optional step)

After sample permeabilization treatment, samples are treated with DNase I (recommended G3342) to prepare positive controls.

2.1. 100 μL of 1×DNase I Buffer (preparation: take 10 μL of 10×DNase I Buffer, then add 90 μL of deionized water and mix well) is added dropwise to the permeabilized samples and incubated for 5 min at room temperature;

2.2. Gently remove the excess liquid, add 100 μL of working solution containing DNase I (20 U/mL) (preparation method: take 10 μL of 10× DNase I Buffer, then add 2 μL of DNase I, and then add 88 μL of deionized water to mix), and incubate for 10 min at room temperature;

2.3. Gently remove excess liquid and wash the slides thoroughly 3-4 times in a staining vat filled with PBS.

Note: Positive control slides must be stained using a separate staining vat, otherwise residual DNase I on the positive control slides may introduce a high background on the experimental slides.

3. Marking and detection

3.1. **Equilibration:** Add 50 μL Equilibration Buffer to each sample to cover all the sample area and incubate for 10 min at room temperature;

3.2. **Labeling solution preparation**: Thaw EdUTP Labeling Mix and Equilibration Buffer on ice and mix enough TdT incubation buffer for all experiments according to the ratio of Recombinant TdT enzyme: EdUTP Labeling Mix: Equilibration Buffer=1 μL:5 μL:50 μL (1:5:50), the volume of reagents used in the specific experiments can be adjusted in equal proportions according to the size of the slides;

3.3. **Negative control system**: Prepare a control TdT incubation buffer without Recombinant TdT enzyme and replace it with ddH2O;

3.4. **Labeling**: Remove as much Equilibration Buffer as possible, then add 56 μL of TdT Incubation

Buffer to each tissue sample and incubate at 37°C for 1 h; be careful not to dry the slides;

3.5. Wash the tissue samples immediately with PBS for 4 washes of 5 min each;

3.6. **Click reaction**: Remove the PBS buffer from the previous step, add 100 μL of click reaction solution dropwise on the sample to cover the sample, incubate for 30 min at room temperature away from light; (refer to the following table for the click reaction system, add each reagent in turn, mixing well while adding, and the amount of preparation can be increased or decreased proportionally, and it is recommended to prepare it beforehand)

Component	Volume
Reaction Buffer A	925 μL
Reaction Buffer B	10 μL
iF488 azide dye	15 μL
Reaction additive （Reagent C）	50 μL
Total volume	1000 μL

3.7. Remove the click reaction solution and immediately wash with PBS buffer 2-3 times for 5 min each;

3.8. Gently wipe off the PBS solution around the sample with filter paper;

3.9. **Nuclear staining**: Samples are stained in a staining vat, where slides are immersed in the dark into a staining vat containing DAPI solution (freshly prepared and diluted with PBS) and left at room temperature for 8 min (or nuclear staining is performed with Hoechst 33258);

3.10. **Sealing**: After the samples are stained, the tissue samples are washed three times with PBS for 5 min each time, then the excess liquid is gently removed and the samples are sealed with drops of anti-fluorescence quenching sealer (recommended G1401);

3.11. **Microscopy**: Immediately analyze the samples under a fluorescence microscope, slides are protected from light, DAPI stains both apoptotic and non-apoptotic cells blue, and only in the nuclei of apoptotic cells there is a green fluorescence localized by iF488 azide dye admixture.

Servicebio® DAB (SA-HRP) Tunel Cell Apoptosis Detection Kit

Cat. #：G1507

Product information

product name	Identification of product	model
DAB (SA-HRP) Tunel Cell Apoptosis Detection Kit	G1507-50T	50T
	G1507-100T	100T

Descirption/Introduction

The breakage of chromosomal DNA in apoptosis is a gradual process. Chromosomal DNA is first degraded into large fragments of 50-300 KB under the action of endogenous nuclease hydrolase, and then about 30% of chromosomal DNA is randomly cut between nucleosome units under the action of ca2+ and mg2+ dependent endonuclease to form 180-200 BP nucleosome DNA polymer. Therefore, in the late stage of apoptosis, DNA will be degraded into 180-200 BP fragments, and a large number of 3'-oh terminals are exposed on the broken genomic DNA. Terminal deoxyribonucleotidyl transferase (TDT) is a template independent DNA polymerase, which can catalyze the binding of deoxynucleotides to the 3'-oh terminal of broken DNA molecules. Therefore, TUNEL (TDT mediated dUTP nick end labeling) cell apoptosis detection kit can be used to detect the nuclear DNA breakage of tissue cells in the late process of apoptosis. The principle is that under the action of TDT enzyme, biotin labeled dUTP (biotin dUTP) is added to the 3´-OH terminal exposed during genomic DNA breakage, and then streptavidin (SA HRP) labeled with horseradish peroxidase (HRP) is used to detect the biotin labeled DNA terminal. Finally, the color reaction is carried out by adding the substrate mixture (DAB) of HRP, the nuclei of apoptotic cells were stained brown, which could be detected by general optical microscope. The kit has a wide range of applications, and is suitable for the detection of apoptosis in paraffin tissue sections, frozen tissue sections, cell climbing slides, cell smears, etc.

Storage and Handling Conditions

Wet ice transportation;

Stored at -20 ℃ , valid for 12 months.

Component

Component Number	Component	G1507-50T	G1507-100T
1507-1	Recombinant TdT Enzyme	50 μL	2×50 μL
1507-2	Biotin-dUTP Labeling Mix	250 μL	2×250 μL
1507-3	Equilibration Buffer	5x1 mL	10×1 μL
1507-4	Streptavidin-HRP	25 μL	2×25 μL
1507-5	Proteinase K（200 μg/mL）	1 mL	2×1 mL
Manual		1 pc	

Preparation

1. PBS phosphate buffer (recommended G0002).
2. Stationary solution: 4% paraformaldehyde dissolved in PBS, pH 7.4 (recommended G1101).
3. Membrane breaker: 0.1%-0.5% Triton X-100 (G1204 recommended).
4. Hydrogen peroxide sealing solution: 3% H_2O_2, prepared with PBS.
5. If you need to dye nuclei, you need to prepare hematoxylin dye solution (recommended G1004) and

hematoxylin differentiation solution (recommended G0139) Hematoxylin bluing solution (recommended G1004)

6. Self prepared DAB color reagent (recommended G1212), n-butanol, xylene, neutral gum, etc
7. Please wear test clothes and disposable gloves during operation.

Assay Protocol / Procedures

1. Sample preparation

A. Paraffin embedded tissue section

1) Paraffin tissue sections were immersed in xylene for 5-10 min at room temperature and repeated 3 times; Then soak in absolute ethanol for 5 min and repeat twice; Finally, it was immersed in gradient ethanol (85%, 75%) and double-distilled water once each for 5 min;

2) Gently moisten the slices with PBS and remove the excess liquid around the sample. Use a PAP Pen to draw a small circle 2-3 mm apart from the tissue along the peripheral contour of the tissue, so as to facilitate downstream permeability treatment and balance marking. During the experiment, do not let the sample dry, and put the treated sample in the wet box to keep the sample moist;

3) Prepare Proteinase K working solution: dilute Proteinase K (200 μg/ml) stock solution with PBS as diluent at the volume ratio of 1:9, so that the final concentration is 20 μg/mL;

4) Add 100 μL Proteinase K working solution drops to each sample, completely covered the tissue and incubated at 37℃ for 20 min;

Note: Proteinase K treatment is mainly helpful for the permeability of staining reagents in subsequent steps of tissues and cells. Too long or too short incubation time will affect the subsequent labeling efficiency. In order to obtain better results, the incubation time of Proteinase K can be optimized.

5) Soak and clean the sample with PBS solution for 3 times, each time for 5 min (Proteinase K needs to be cleaned, otherwise it will interfere with the subsequent marking reaction). The treated sample is placed in a wet box to keep the sample moist;

6) (optional steps) remove the excess liquid from the sample, add an appropriate amount of membrane breaking liquid to the tissue, fully infiltrate the tissue, and treat at room temperature for 20 min; After the membrane breaking treatment is completed, the sample shall be moistened with PBS solution for 3 times for 5 min each time; The treated sample is placed in a wet box to keep the sample moist;

7) Remove the excess liquid from the sample, drop an appropriate amount of 3% H_2O_2 (prepared with PBS) onto the tissue, fully infiltrate the tissue, and treat it at room temperature for 20 min (inactivate the endogenous peroxidase in the tissue, and the incubation time should not be too long, otherwise DNA breakage caused by hydrogen peroxide will occur, resulting in false positive); Then moisten the sample with PBS solution for 3 times, each time for 5 min; The treated sample is placed in a wet box to keep the sample moist.

B. Tissue frozen section

1) Immerse the slide in 4% paraformaldehyde solution (dissolved in PBS) for fixation, and incubate at room temperature for 10-15 min;

2) After the film is removed from the fixing liquid, it shall be naturally dried in the fume hood;

3) Put the slide into pure water or PBS for moistening and washing, and remove the residual fixed liquid on the slide;

4) Draw a small circle 2-3 mm apart from the tissue along the peripheral contour of the tissue with a PAP Pen to facilitate downstream permeability treatment and balance marking; During the experiment, do not let the sample dry, and put the treated sample in the wet box to keep the sample

moist;

5) Prepare Proteinase K working solution: dilute Proteinase K (200 μg/ml) stock solution with PBS as diluent at the volume ratio of 1:9, so that the final concentration is 20 μg/mL;

6) Add 100 μL Proteinase K working solution drops to each sample, completely covered the tissue and incubated at 37 ℃ for 20 min;

Note: Proteinase K treatment is mainly helpful for the permeability of staining reagents in subsequent steps of tissues and cells. Too long or too short incubation time will affect the subsequent labeling efficiency. In order to obtain better results, the incubation time of Proteinase K can be optimized.

7) Moisten the sample with PBS solution for 2-3 times to remove the excess liquid (Proteinase K needs to be cleaned, otherwise it will interfere with the subsequent marking reaction). The treated sample is placed in a wet box to keep the sample moist;

8) ***(optional steps)*** add an appropriate amount of membrane breaking liquid to the tissue, fully infiltrate the tissue, and treat it at room temperature for 20 min. after the membrane breaking treatment, similarly wash the sample with PBS solution to remove the excess liquid. The treated sample is placed in a wet box to keep the sample moist;

9) Remove the excess liquid from the sample, drop an appropriate amount of 3% H_2O_2 (prepared with PBS) onto the tissue, fully infiltrate the tissue, and treat it at room temperature for 20 min (inactivate the endogenous peroxidase in the tissue, and the incubation time is not easy to be too long, otherwise DNA breakage caused by hydrogen peroxide will occur, resulting in false positive); Then moisten the sample with PBS solution for 3 times, each time for 5 min; The treated sample is placed in a wet box to keep the sample moist.

C. Cell climbing sheet

1) Adherent cells were cultured on lab Tek. After apoptosis induction treatment, the slides were gently rinsed twice with PBS;

2) Add an appropriate amount of 4% paraformaldehyde solution (dissolved in PBS) to each slide chamber for fixation, and incubate at room temperature for 20 min;

3) Remove the stationary solution and add PBS for cleaning for 3 times, each time for 5 min;

4) Each sample was immersed in membrane breaking solution and incubated at room temperature for 5 min for permeability treatment(Note: It is recommended to use 2-20 μg/mL of Proteinase K working solution for digestion, and treat at 37℃ for about 10 min, depending on the cell status. If the cells are prone to drop off, it is recommended to treat them with membrane-breaking solution);

5) Immerse the cleaning sample in an open beaker containing PBS solution for 2-3 times; Add an appropriate amount of 3% H_2O_2 (prepared with PBS) to each slide chamber and treat it at room temperature for 20 min;

6) Gently remove the excess liquid, add PBS for cleaning for 3 times, 5 min each time; Carefully dry the liquid around the sample on the slide with filter paper. The treated sample is placed in a wet box to keep the sample moist.

D. Cell smear

1) Cells were resuspended in PBS at a concentration of approximately 2×10^7 cells/mL, and 50-100 μL of cell suspension was aspirated onto an anti-debonding slide, and a clean slide was used to gently spread the cell suspension;

2) Immerse the glass slides in a staining tank containing 4% of the fresh paraformaldehyde prepared in PBS, fix the cells, and place them at 4 ℃ for 25 min;

3) Immerse the slide in PBS, place it at room temperature for 5 min, and wash it again;

4) Each sample was immersed in membrane breaking solution and incubated at room temperature for 5 min for permeability treatment (Note: It is recommended to use 2-20 μg/mL of Proteinase K working solution for digestion, and treat at 37℃ for about 10 min, depending on the cell status. If the cells are easy to fall off, it is recommended to use the membrane-breaking solution for treatment);

5) Immerse the cleaning sample in an open beaker containing PBS solution for 2-3 times; Add an appropriate amount of 3% H_2O_2 (prepared with PBS) to each sample and treat it at room temperature for 20 min;

6) Gently remove the excess liquid, add PBS for cleaning for 3 times, 5 min each time; Finally, carefully dry the liquid around the sample on the slide with filter paper. The treated sample is placed in a wet box to keep the sample moist.

2. DNase I treatment positive control experiment (optional steps)

After the sample permeability treatment, treat the sample with DNase I (recommended G3342) to prepare the positive control.

1) Add 100 μL 1 × DNase I buffer (preparation method: take 10 μL 10 × DNase I buffer, and then add 90 μL deionized water mixed evenly) to the permeable sample and incubated at room temperature for 5 min;

2) Gently remove excess liquid and add 100 μL working liquid containing DNase I (20 U/ml), incubate at room temperature for 10 min;

3) Gently remove the excess liquid, and thoroughly wash the slide 3-4 times in the staining tank with PBS;

(Note: the positive control slide must use a separate staining cylinder, otherwise the residual DNase I on the positive control slide may introduce a high background on the experimental slide).

3. Marking and testing

1) Balance: add 50 μL equip buffer drops per sample to cover all the sample areas to be tested, and incubate at room temperature for 10 min;

2) Preparation of labeling solution: thaw the biotin dUTP labeling mix and equivalence buffer on ice, and mix sufficient TDT incubation buffer for all experiments according to the proportion of recombinant TDT enzyme:biotin dUTP labeling mix:equivalence buffer=1 μL:5 μLl:50 μL (1:5:50). The volume of the reagent used in the specific experiment can be adjusted according to the size of the slide;

3) Negative control system: prepare a control TDT incubation buffer without recombinant TDT enzyme and replace it with ddH_2O;

4) Marking: try to remove the balanced equalization buffer, and then add 56 μL TDT incubation buffer to each tissue sample, incubated at 37 ℃ for 1 h; Be careful not to dry the slide, and keep the slide away from light;

5) Immediately moisten and wash the tissue samples with PBS for 4 times, each time for 5 min;

6) Gently wipe the PBS solution around the sample with filter paper;

7) Streptavidin HRP reaction: after drying the slides, each sample tissue was added with 100 μL (infiltrated tissue) streptavidin HRP reaction solution (streptavidin-HRP:TBST=1:200-500 dilution in advance), and incubated at 37 ℃ for 30 min;

8) Clean the sample with PBS for 3 times, 5 min each time;

9) DAB color development: prepare DAB color development working solution (prepared

and used now), G1212 is recommended. Add 50-100 μL DAB color developing working solution to each sample, put the slices under the microscope to observe the color development in real time, put the slices into the wet box immediately after the positive display, and wash them with pure water to terminate the reaction;

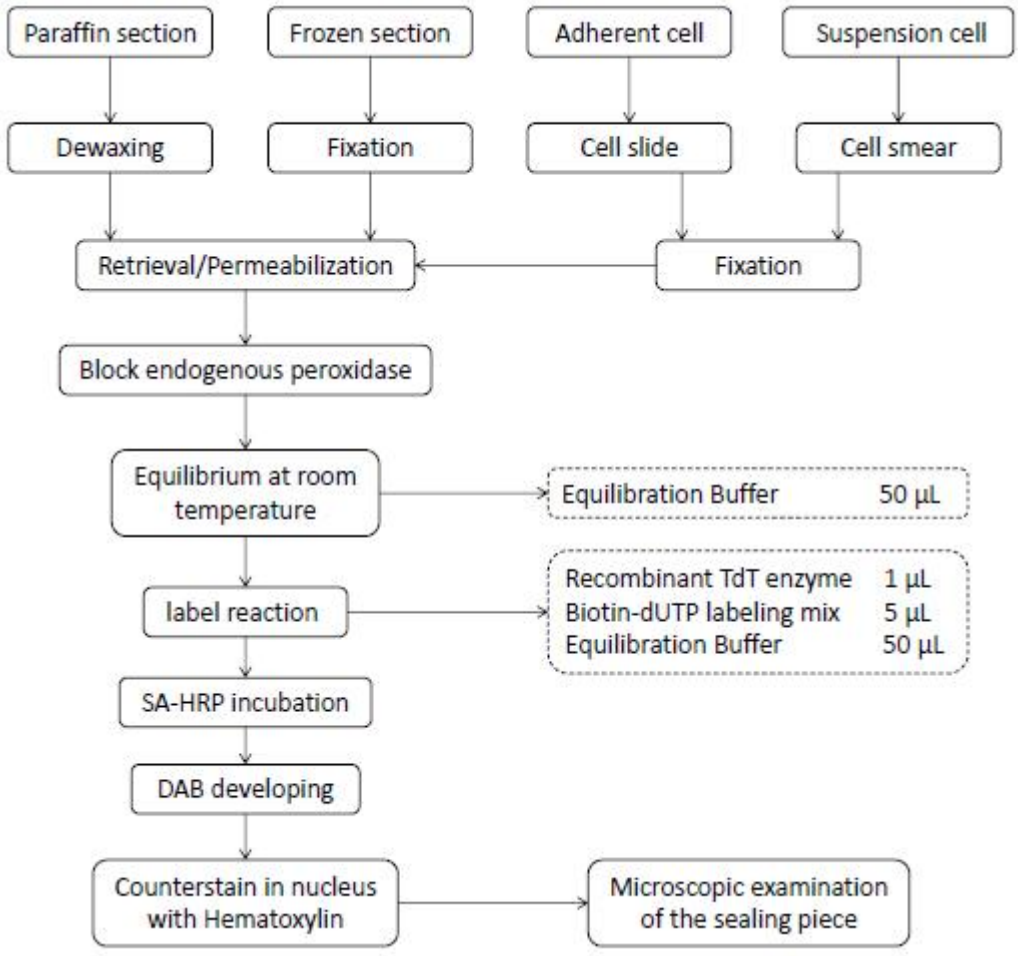
10) Hematoxylin staining nucleus: after the sections are stained with hematoxylin staining solution for 3-5 min, they are immediately washed with pure water. After differentiation with hematoxylin differentiation solution for about 2 s, they are immediately washed with pure water, and then returned to blue with hematoxylin re blue solution for a few seconds. After the staining nucleus is completed, they need to be examined under the microscope. If the staining is too deep, they return to the differentiation solution for re differentiation; if the staining is too light, they can be re stained from the hematoxylin staining nucleus;

11) Transparent: the sample is dehydrated with fresh absolute ethanol for 4 times, each time for 5 min; Soak it in n-butanol for 5 min, put it into xylene for 5 min to be transparent, and replace it with fresh xylene for 5 min to be transparent again;

12) Sealing: use neutral gum to seal the slices and dry them naturally or in an oven at 60 ℃;

13) Microscopic examination: the samples were examined with a white light microscope (the apoptotic positive nuclei were dyed brown, and the normal negative nuclei were dyed blue).

4. Experiment flow diagram



Servicebio® Click-iT 647 Tunel Cell Apoptosis Detection Kit

Cat. No.: G1509-50T

Product Information

Product Name	Cat. No.	Spec.
Click-iT 647 Tunel Cell Apoptosis Detection Kit	G1509-50T	50T

Product Description/Introduction

The breaking of chromosomal DNA in apoptosis is a gradual, phased process. Chromosomal DNA is first degraded into large 50-300 kb segments by endogenous nucleic acid hydrolases, and then about 30% of chromosomal DNA is randomly cut between nucleosome units in the presence of Ca^{2+} and Mg^{2+} dependent nucleic acid endonucleases to form 180-200 bp nucleosomal DNA multimers. Thus, in late apoptosis, DNA is degraded into 180-200 bp fragments, and a large number of 3'-OH ends are exposed on the broken genomic DNA. Terminal Deoxynucleotidyl Transferase (TdT) is a template-independent DNA polymerase that catalyzes the incorporation of deoxyribonucleotides into the 3'-OH ends of broken DNA molecules. Therefore TUNEL (TdT mediated dUTP Nick End Labeling) Cell Apoptosis Detection Kit can be used to detect the breakage of nuclear DNA in tissue cells during late apoptosis.

The principle is that under the action of TdT enzyme, EdUTP (a dUTP with alkyne modification) is doped into the 3´-OH end exposed during genomic DNA breakage, and the alkyne group reacts with the azide dye in a ring-forming reaction catalyzed by a monovalent copper ion (click reaction) thus introducing the fluorescent moiety in a targeted way. iF647 azide is used in this kit, thus it can be detected by fluorescence microscope or flow cytometry (iF647 excitation 656 nm, emission 670 nm). Compared to other modified dUTPs, EdUTP has a smaller spatial site resistance and is more easily doped into DNA ends by TdTase.

This kit has a wide range of applications and is suitable for apoptosis detection in paraffin tissue sections, frozen tissue sections, cell crawls and cell smears.

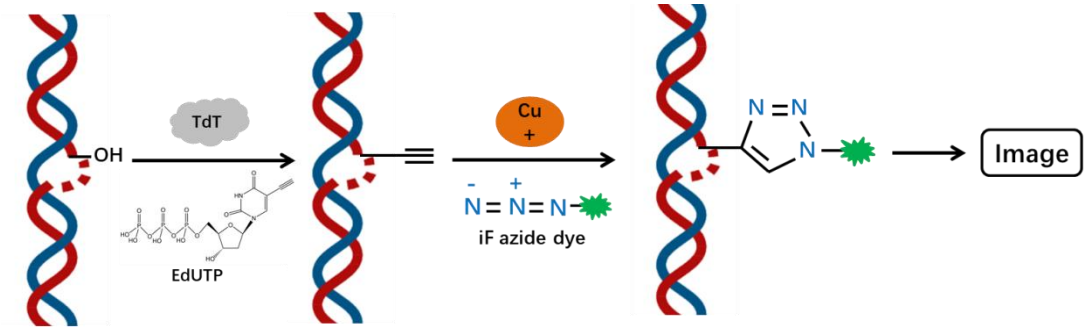


Figure 1. Schematic diagram of the click chemistry-based TUNEL kit principle

Storage and Shipping Conditions

Ship with wet ice; Store at -20℃ for 12 months.

Product Content

Component Number	Component	G1509-50T
G1509-1	Recombinant Tdt Enzyme	50 μL
G1509-2	EdUTP Labeling Mix	250 μL
G1509-3	Equilibration Buffer	5×1 mL

G1509-4	Proteinase K (200μg/mL)	1 mL
G1509-5	iF647 azide dye	80 μL
G1509-6	Reaction Buffer A	5×1 mL
G1509-7	Reaction Buffer B	60 μL
G1509-8	Reaction additive (Reagent C)	2×100 mg
Manual		1 pc

Pre-experimentation

1. PBS phosphate buffer (recommended G0002 or G4202);
2. Fixed solution: 4% paraformaldehyde dissolved in PBS, pH 7.4 (recommended G1101);
3. Membrane-breaking solution: 0.1% Triton X-100 dissolved in 0.1% sodium citrate (recommended G1204);
4. 0.2% Triton X-100 prepared in PBS; 0.1% Triton X-100 prepared in PBS containing 5 mg/mL BSA;
5. For nuclear staining, provide your own DAPI (2 μg/mL), Hoechst 33258, or PI (1 μg/mL) (G1012, G1011, G1021 recommended);
6. DNase I (G3342) for positive control experiments;
7. Reaction additive (Reagent C) is centrifuged at low speed, 100 mg of the powder is dissolved in 1 mL of ultrapure water (ready to use), and 100 μL is dispensed and stored at -20°C, leaving the remaining powder for spare use; (Reagent C is easy to oxidize, try to avoid long time exposure to the air, after formulated into an aqueous solution, it is strongly recommended to divide into small portions for use; After testing the aqueous solution of Reagent C, the color of a slight change, click the reaction system can still be carried out normally, and if it shows brown color, it indicates that the component has been invalidated)
8. If the background color of the result is too dark, it may be caused by insufficient washing and residual fixative during the experiment;
9. Be careful to add the reaction liquid in sequence, and mix well while adding;
10. For your health and safety, please wear lab coat and gloves during operation.

Assay Protocol / Procedures

1. Sample Preparation

A. Paraffin-embedded tissue sections

a. The paraffin tissue sections were immersed in Eco-friendly dewaxing clear solution (G1128) for 5-10 min at room temperature and repeated 3 times; Then soak in anhydrous ethanol for 5 min and repeat twice; Finally, soak in gradient ethanol (85%, 75%, and double-distilled water) once for 5 min each;

b. Gently moisten the section with PBS and remove excess liquid around the sample, use a PAP Pen to draw a small circle spaced 2-3 mm apart from the tissue along the tissue's peripheral contour to facilitate downstream permeability processing and equilibrium labeling operations, do not allow the sample to dry out during experiments, and keep the processed samples in a wet box to keep the samples moist;

c. **Preparation of Proteinase K working solution**: Dilute the Proteinase K (200 μg/mL) stock solution with PBS as diluent at a ratio of 1:9 to give a final concentration of 20 μg/mL;

d. Add 100 μL of the above Proteinase K working solution to each sample to make it fully covered, and incubate at 37°C for 20 min;

Note: Proteinase K treatment mainly contributes to the permeation of staining reagents in the subsequent steps of tissues and cells, and its incubation time is too long or too short to affect the

efficiency of the subsequent labeling, in order to get better results, the incubation time can be optimized according to the actual situation.

e. Wash the samples by moistening with PBS solution 3 times for 5 min each time (Proteinase K needs to be washed clean or it will interfere with the subsequent labeling reaction). The treated samples were placed in a wet box to keep the samples moist;

f. (Optional step) Remove the excess liquid on the sample, add appropriate amount of membrane-breaking solution droplets to the tissue, fully infiltrate the tissue, and treat it for 20 min at room temperature; after the membrane-breaking treatment is completed, the sample is similarly washed with PBS solution for 3 times, each time for 5 min; the treated sample is placed in a wet box to keep the sample moist.

B. Tissue frozen section

a. The tissue frozen sections are submerged in the fixative and incubated and fixed for 10-15 min at room temperature;

b. The tissue sections are removed from the fixative and placed in a fume hood to dry naturally;

c. Tissue sections are moistened and washed in purified water or PBS to remove residual fixative from the sample;

d. Use a histochemical pen to draw a small circle spaced 2-3 mm apart from the tissue along the tissue's peripheral contour to facilitate downstream permeability processing and equilibrium labeling operations, do not allow the sample to dry out during experiments, and keep the processed samples in a wet box to keep the samples moist;

e. Preparation of Proteinase K working solution: Dilute the Proteinase K (200 μg/mL) stock solution with PBS as diluent at a ratio of 1:9 to give a final concentration of 20 μg/mL;

f. Add 100 μL of the above Proteinase K working solution to each sample to make it fully covered, and incubate for 10 min at room temperature;

Note: Proteinase K treatment mainly contributes to the permeation of staining reagents in the subsequent steps of tissues and cells, and its incubation time is too long or too short to affect the efficiency of the subsequent labeling, in order to get better results, the incubation time can be optimized according to the actual situation.

g. Wash the sample 2-3 times with PBS solution to remove excess liquid (Proteinase K needs to be washed clean or it will interfere with the subsequent labeling reaction), and keep the treated sample in a wet box to keep the sample moist;

h. (Optional step) Add appropriate amount of membrane-breaking solution droplets to the tissue, fully infiltrate the tissue, room temperature treatment for 20 min, membrane-breaking treatment is completed the same after the completion of the sample with the PBS solution to rinse, to remove the excess liquid, after the treatment of the sample in the wet box to keep the sample wet.

C. Cell creep

a. Adherent cells are cultured on Lab-Tek slide chambers (Chamber Slides), and slides are gently moistened and washed 2 times with PBS after apoptosis induction treatment;

b. Appropriate amount of fixative is added to each slide chamber to cover the tissue and incubated for 20 min at room temperature;

c. Remove the fixative and add PBS to wash 3 times for 5 min each;

d. Each sample is immersed in 0.2% Triton X-100 solution prepared in PBS and incubated for 5 min at room temperature for permeabilization;

e. Wash samples by submerging them 2-3 times in an open beaker containing PBS solution;

f. Gently remove excess liquid and use filter paper to carefully blot the liquid around the sample on the slide.The processed sample is placed in a wet box to keep the sample moist.

D. Cell smear

a. Cells are resuspended in PBS at a concentration of about 2 × 10^7 cells/mL, and 50-100 μL of cell suspension is aspirated onto an anti-detachment slide, and a clean slide is used to gently spread the cell suspension;

b. The cell smears are immersed in a staining jar filled with fixing solution, and the cells are fixed and left at 4°C for 25 min;

c. Immerse the slide in PBS and leave it at room temperature for 5 min to soak and rinse, repeat once;

d. Gently remove the excess liquid and carefully blot the excess liquid around the sample on the slide with filter paper, draw a small circle along the peripheral contour of the cell with a histochemical pen to facilitate downstream permeability processing and equilibrium labeling operations, and do not allow the sample to dry out during the course of the experiment;

e. Each sample is immersed in 0.2% Triton X-100 solution prepared in PBS and incubated for 5 min at room temperature for permeabilization;

f. Wash samples by submerging them 2-3 times in an open beaker containing PBS solutio

g. Gently remove excess liquid and carefully blot the liquid around the sample on the slide with filter paper. Keep the sample moist by placing the treated sample in a wet box.

2. DNase I treatment positive control experiment (optional step)

After sample permeabilization treatment, samples are treated with DNase I (recommended G3342) to prepare positive controls.

2.1. 100 μL of 1×DNase I Buffer (preparation: take 10 μL of 10×DNase I Buffer, then add 90 μL of deionized water and mix well) is added dropwise to the permeabilized samples and incubated for 5 min at room temperature;

2.2. Gently remove the excess liquid, add 100 μL of working solution containing DNase I (20 U/mL) (preparation method: take 10 μL of 10× DNase I Buffer, then add 2 μL of DNase I, and then add 88 μL of deionized water to mix), and incubate for 10 min at room temperature;

2.3. Gently remove excess liquid and wash the slides thoroughly 3-4 times in a staining vat filled with PBS.

Note: Positive control slides must be stained using a separate staining vat, otherwise residual DNase I on the positive control slides may introduce a high background on the experimental slides.

3. Marking and detection

3.1. **Equilibration:** Add 50 μL Equilibration Buffer to each sample to cover all the sample area and incubate for 10 min at room temperature;

3.2. **Labeling solution preparation**: Thaw EdUTP Labeling Mix and Equilibration Buffer on ice and mix enough TdT incubation buffer for all experiments according to the ratio of Recombinant TdT enzyme: EdUTP Labeling Mix: Equilibration Buffer=1 μL:5 μL:50 μL (1:5:50), the volume of reagents used in the specific experiments can be adjusted in equal proportions according to the size of the slides;

3.3. **Negative control system**: Prepare a control TdT incubation buffer without Recombinant TdT enzyme and replace it with ddH2O;

3.4. **Labeling**: Remove as much Equilibration Buffer as possible, then add 56 μL of TdT Incubation

Buffer to each tissue sample and incubate at 37°C for 1 h; be careful not to dry the slides;

3.5. Wash the tissue samples immediately with PBS for 4 washes of 5 min each;

3.6. **Click reaction**: Remove the PBS buffer from the previous step, add 100 μL of click reaction solution dropwise on the sample to cover the sample, incubate for 30 min at room temperature away from light; (refer to the following table for the click reaction system, add each reagent in turn, mixing well while adding, and the amount of preparation can be increased or decreased proportionally, and it is recommended to prepare it beforehand)

Component	Volume
Reaction Buffer A	925 μL
Reaction Buffer B	10 μL
iF647 azide dye	15 μL
Reaction additive （Reagent C）	50 μL
Total volume	1000 μL

3.7. Remove the click reaction solution and immediately wash with PBS buffer 2-3 times for 5 min each;

3.8. Gently wipe off the PBS solution around the sample with filter paper;

3.9. **Nuclear staining**: Samples are stained in a staining vat, where slides are immersed in the dark into a staining vat containing DAPI solution (freshly prepared and diluted with PBS) and left at room temperature for 8 min (or nuclear staining is performed with Hoechst 33258);

3.10. **Sealing**: After the samples are stained, the tissue samples are washed three times with PBS for 5 min each time, then the excess liquid is gently removed and the samples are sealed with drops of anti-fluorescence quenching sealer (recommended G1401);

3.11. **Microscopy**: Immediately analyze the samples under a fluorescence microscope, slides are protected from light, DAPI stains both apoptotic and non-apoptotic cells blue, and only in the nuclei of apoptotic cells there is a pink fluorescence localized by iF647 azide dye admixture.

Servicebio® Annexin V-EGFP/PI Cell Apoptosis Detection Kit (Green EGFP + Red PI)

Cat #: G1510

Product Information

Product Name	Cat. No	Spec.
Annexin V-EGFP/PI Cell Apoptosis Detection Kit (Green EGFP + Red PI)	G1510-50T	50 T
	G1510-100T	100 T

Product Description/Introduction

Apoptosis is a normal physiological process that occurs during embryonic development and maintenance of tissue homeostasis and is accompanied by many morphological changes, among which the loss of cell membrane is one of the early characteristics of apoptosis. In normal cells, phosphatidylserine (PS) is only distributed on the inner side of the phospholipid bilayer of the cell membrane. However, in the early stage of apoptosis, PS flips from the inner side of the lipid membrane to the outer side, exposing it to the outside of the cell. Annexin V is a Ca^{2+}-dependent phospholipid-binding protein with high affinity for PS and it can specifically bind to cells exposed to PS. Therefore, Annexin V is used as one of the indicators to detect early apoptosis of cells. Propidium iodide (PI) is a nucleic acid dye that cannot penetrate normal cells with intact cell membranes and early apoptotic cells, but it can penetrate the cell membranes of late apoptotic and necrotic cells and stain cell nuclei.

This product uses a fusion protein composed of EGFP (enhanced Green Fluorescent Protein) and Annexin V as a detection probe to detect early apoptosis of cells. The PI is also used to distinguish live cells from necrotic and late apoptotic cells. In combination with Annexin V-EGFP and PI, live cells show negative staining (Annexin V-/PI-), early apoptotic cells show single fluorescence positive (Annexin V+/PI-), while late apoptotic and necrotic cells show double fluorescence positive (Annexin V+/PI+). This kit is suitable for flow cytometry or fluorescence microscopy detection. It is also suitable for quantitative detection of apoptotic cells as EGFP is fused 1:1 to Annexin V.

Storage and Shipping Conditions

Ship with wet ice; Store at 2-8℃ away from light, valid for 12 months.

Product Components

Component Number	Component	G1510-50T	G1510-100T
G1510-1	Annexin V-EGFP	250 μL	2×250 μL
G1510-2	Propidium Iodide (PI)	250 μL	2×250 μL
G1510-3	1×Binding Buffer	25 mL	2×25 mL
Manual		1 pc	

Product Protocol/Procedures

1. Suspension of cells: Take cell suspension and collect the cells by centrifugation at 500 x g for 5 min at 4℃.

Adherent cells: Collect cells culture supernatant first. then digest cells with trypsin without EDTA (recommended G4002 or G4011), combine with the cell culture supernatant and collect the cells by centrifugation at 500 x g for 5 min at 4℃. Trypsin digestion should not be too long to avoid false positives caused by excessive digestion.

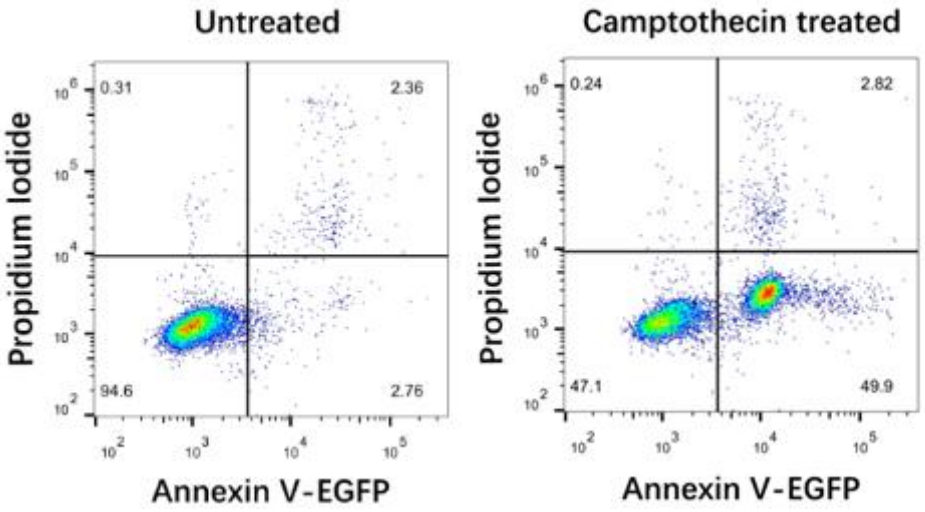
2. Wash the cells twice with pre-cooled PBS (recommended G4202), and collect the cells by centrifugation at 500 x g for 5 min at 4℃ each time.
3. Gently resuspend the cells with pre-cooled 1×Binding Buffer, and adjust the cell concentration to 1~5 $\times 10^6$/mL.
4. Add 5 μL of Annexin V-EGFP and 5 μL of PI to 100 μL of cell suspension, mix gently and protect from light at room temperature for 8-10 min.
5. Add 400 μL of pre-cooled 1×Binding Buffer, shake gently, and use flow cytometry or fluorescence microscope for detection within 1 hour.

Result Analysis

1. Flow Cytometry Detection

a) Select the appropriate voltage and adjust the light compensation for the flow cytometer analysis. it is recommended to set a negative control (without Annexin V-EGFP and PI labeling) to adjust the voltage except for the experimental group, and the single standard control (with Annexin V-EGFP only, and cells with PI only) for compensation adjustment.

b) Reference example of flow cytometry detection and analysis: Induce Jurkat T lymphoma cells with 5 μM Camptothecin for 6 h. Referring to the above experimental steps, use flow cytometry to detect. The results are shown in the following figure.



The maximum excitation wavelength of EGFP is 488 nm and the maximum emission wavelength is 507 nm; the maximum excitation wavelength of PI-DNA complex is 535 nm and the maximum emission wavelength is 615 nm. A two-colour scatter plot is plotted by flow cytometry correlation analysis software, with EGFP in the horizontal coordinate and PI in the vertical coordinate. In a typical experiment, live cells are non-fluorescent and the scatter point is located in the lower left first quadrant. Cells in early apoptosis have a strong green fluorescence and the scatter is in the second lower right quadrant. Late stage apoptotic and necrotic cells show dual red and green fluorescence, with the scatter point located in the upper right third quadrant.

2. Fluorescence Microscopy Detection

a) Add 5-10 μL of Annexin V-EGFP and PI double-stained cell suspension to the slide.

b) Cover with a coverslip.

c) Observe with a two-color filter under a fluorescence microscope. Annexin V-EGFP has a green fluorescence signal, and PI has a red fluorescence signal **(**When taking pictures with a fluorescence microscope, it is recommended to add an appropriate amount of anti-fluorescence quenching sealer (G1401) to prevent fluorescence quenching problems).

Note

1. The entire experimental process should be handled as gently as possible to avoid cell fragmentation, which may affect the experimental results.
2. Washing the cells with PBS cannot be omitted, and the residual PBS should also be removed as much as possible.
3. When using trypsin to digest cells, the experiment should be handled carefully and the digestion time should be controlled to avoid artificial damage to the cells. If the digestion time is too short, the cells need to be vigorously beaten to fall off, which may easily cause mechanical damage to the cell membrane; if the digestion time is too long, the cell membrane is also easily damaged and the results will be affected. In addition, trypsin containing EDTA cannot be used. EDTA will affect the binding of Annexin V to PS.
4. If some cells are floating after apoptosis stimulation, collect both the cell culture supernatant and the adherent cells to stain for a more accurate result.
5. Annexin V-EGFP and PI are sensitive to light, please avoid light during operation. Testing should be performed as soon as possible after the reaction is complete.
6. For your safety and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® Annexin V-FITC/PI Cell Apoptosis Detection Kit (Green FITC + Red PI)

Cat #: G1511

Product Information

Product Name	Cat. No	Spec.
Annexin V-FITC/PI Cell Apoptosis Detection Kit (Green FITC + Red PI)	G1511-50T	50 T
	G1511-100T	100 T

Product Description/Introduction

Apoptosis is a normal physiological process that occurs during embryonic development and maintenance of tissue homeostasis and is accompanied by many morphological changes, among which the loss of cell membrane is one of the early characteristics of apoptosis. In normal cells, phosphatidylserine (PS) is only distributed on the inner side of the phospholipid bilayer of the cell membrane. However, in the early stage of apoptosis, PS will flip from the inner side of the lipid membrane to the outer side, exposing it to the outside of the cell. Annexin V is a Ca^{2+}-dependent phospholipid-binding protein with high affinity for PS and it can specifically bind to cells exposed to PS. Therefore, Annexin V is used as one of the indicators to detect early apoptosis of cells. Propidium iodide (PI) is a nucleic acid dye that cannot penetrate normal cells with intact cell membranes and early apoptotic cells, but it can penetrate the cell membranes of late apoptotic and necrotic cells and stain cell nuclei.

This product uses a fusion protein composed of FITC and Annexin V as a detection probe to detect early apoptosis of cells. The PI is also used to distinguish surviving cells from necrotic and late apoptotic cells. In combination with Annexin V-FITC and PI, live cells show negative staining (Annexin V^-/PI^-), early apoptotic cells show single fluorescence positive (Annexin V^+/PI^-), while late apoptotic and necrotic cells show double fluorescence positive (Annexin V^+/PI^+). This kit is suitable for flow cytometry or fluorescence microscopy detection.

Storage and Shipping Conditions

Ship with wet ice; Store at 2-8℃ away from light, valid for 12 months.

Product Components

Component Number	Component	G1511-50T	G1511-100T
G1511-1	Annexin V-FITC	250 μL	2×250 μL
G1511-2	Propidium Iodide (PI)	250 μL	2×250 μL
G1511-3	1×Binding Buffer	25 mL	2×25 mL
Manual		1 pc	

Product Protocol/Procedures

1. Suspension of cells: Take the cell suspension and collect the cells by centrifugation at 500 x g for 5 min at 4℃.

Adherent cells: Collect cells culture supernatant first. then digest cells with trypsin without EDTA (recommended G4002 or G4011), combine with the cell culture supernatant and collect the cells by centrifugation at 500 x g for 5 min at 4℃. Trypsin digestion should not be too long to avoid false positives caused by excessive digestion.

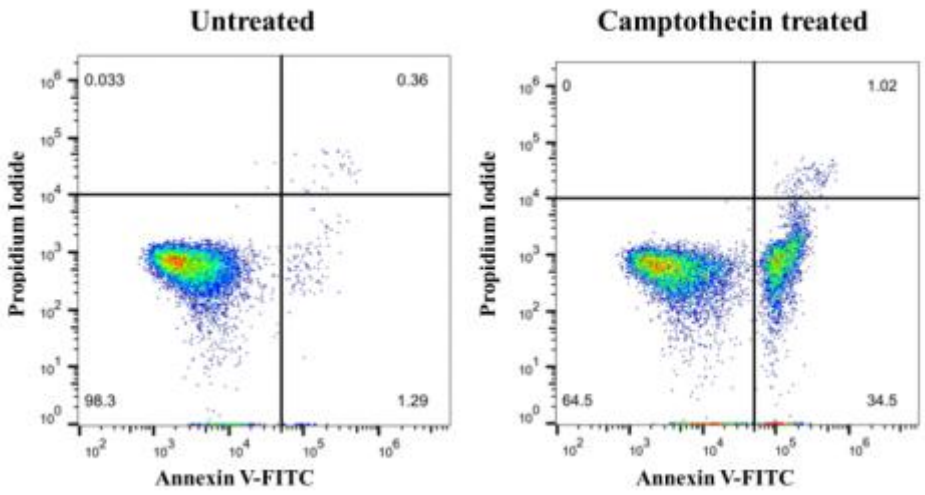
2. Wash the cells twice with pre-cooled PBS (recommended G4202), and collect the cells by centrifugation at 500 x g for 5 min at 4℃ each time.
3. Gently resuspend the cells with pre-cooled 1×Binding Buffer, and adjust the cell concentration to 1~5 $\times 10^6$/mL.
4. Add 5 μL of Annexin V-EGFP and 5 μL of PI to 100 μL of cell suspension, mix gently and protect from light at room temperature for 8-10 min.
5. Add 400 μL of pre-cooled 1×Binding Buffer, shake gently, and use flow cytometry or fluorescence microscope for detection within 1 hour.

Result Analysis

1. Flow Cytometry Detection

a) Select the appropriate voltage and adjust the light compensation for the flow cytometer analysis. it is recommended to set a negative control (without Annexin V-FITC and PI labeling) to adjust the voltage except for the experimental group, and the single standard control (with Annexin V-FITC only, and cells with PI only) for compensation adjustment.

b) Reference example of flow cytometry detection and analysis: Induce Jurkat T lymphoma cells with 5 μM Camptothecin for 6 h. Referring to the above experimental steps, use flow cytometry to detect. The results are shown in the following figure.



The maximum excitation wavelength of FITC is 488 nm and the maximum emission wavelength is 525 nm; the maximum excitation wavelength of PI-DNA complex is 535 nm and the maximum emission wavelength is 615 nm. A two-colour scatter plot is plotted by flow cytometry correlation analysis software, with FITC in the horizontal coordinate and PI in the vertical coordinate. In a typical experiment, live cells are non-fluorescent and the scatter point is located in the lower left first quadrant. Cells in early apoptosis have a strong green fluorescence and the scatter is in the second lower right quadrant. Late stage apoptotic and necrotic cells show dual red and green fluorescence, with the scatter point located in the upper right third quadrant.

2. Fluorescence Microscopy Detection

a) Add 5-10 μL of Annexin V-FITC and PI double-stained cell suspension to the slide.
b) Cover with a coverslip.
c) Observe with a two-color filter under a fluorescence microscope. Annexin V-FITC has a green fluorescence signal, and PI has a red fluorescence signal. (When taking pictures with a fluorescence microscope, it is recommended to add an appropriate amount of anti-fluorescence quenching sealer (G1401) to prevent fluorescence quenching problems)

Note

1. The entire experimental process should be handled as gently as possible to avoid cell fragmentation, which may affect the experimental results.
2. Washing the cells with PBS cannot be omitted, and the residual PBS should also be removed as much as possible.
3. When using trypsin to digest cells, the experiment should be handled carefully and the digestion time should be controlled to avoid artificial damage to the cells. If the digestion time is too short, the cells need to be vigorously beaten to fall off, which may easily cause mechanical damage to the cell membrane; if the digestion time is too long, the cell membrane is also easily damaged and the results will be affected. In addition, trypsin containing EDTA cannot be used. EDTA will affect the binding of Annexin V to PS.
4. If some cells are floating after apoptosis stimulation, collect both the cell culture supernatant and the adherent cells to stain for a more accurate result.
5. Annexin V-FITC and PI are sensitive to light, please avoid light during operation. Testing should be performed as soon as possible after the reaction is complete.
6. For your safety and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® Annexin V-PE/7-AAD Cell Apoptosis Detection Kit

Cat #: G1512

Product Information

Product Name	Cat. No	Spec.
Annexin V-PE/7-AAD Cell Apoptosis Detection Kit	G1512-50T	50 T
	G1512-100T	100 T

Product Description/Introduction

Apoptosis is a normal physiological process that occurs during embryonic development and maintenance of tissue homeostasis and accompanied by many morphological changes, among which the loss of cell membrane is one of the early characteristics of apoptosis. In normal cells, phosphatidylserine (PS) is only distributed on the inner side of the phospholi7-ADDd bilayer of the cell membrane. However, in the early stage of apoptosis, PS will flip from the inner side of the li7-ADDd membrane to the outer side, exposing it to the outside of the cell. Annexin V is a Ca^{2+}-dependent phospholi7-ADDd-binding protein with high affinity for PS and it can specifically bind to cells exposed to PS. Therefore, Annexin V is used as one of the indicators to detect early apoptosis of cells. Pro7-ADDdium iodide (7-ADD) is a nucleic acid dye that cannot penetrate normal cells with intact cell membranes and early apoptotic cells, but it can penetrate the cell membranes of late apoptotic and necrotic cells and stain cell nuclei.

This product uses a fusion protein composed of PE (Phycoerythrin) and Annexin V as a detection probe to detect early apoptosis of cells. The 7-AAD is used to distinguish surviving cells from necrotic and late apoptotic cells. In combination with Annexin V-PE and 7-ADD, live cells showed negative staining (Annexin $V^-/7\text{-}ADD^-$), early apoptotic cells showed single fluorescence positive (Annexin $V^+/7\text{-}ADD^-$), and late apoptotic cells and necrotic cells show double fluorescence positive (Annexin $V^+/7\text{-}ADD^+$). This kit is suitable for flow cytometry or fluorescence microscopy. It is also suitable for quantitative detection of apoptotic cells as PE is fused 1:1 to Annexin V.

Storage and Shipping Conditions

Ship with wet ice; Store at 2-8℃ away from light, valid for 12 months.

Product Components

Component Number	Component	G1512-50T	G1512-100T
G1512-1	Annexin V-PE	250 μL	2×250 μL
G1512-2	7-AAD	250 μL	2×250 μL
G1512-3	1×Binding Buffer	25 mL	2×25 mL
Manual		1 pc	

Product Protocol/Procedures

1. Suspension of cells: Take the cell suspension and collect the cells by centrifugation at 500 x g for 5 min at 4℃.

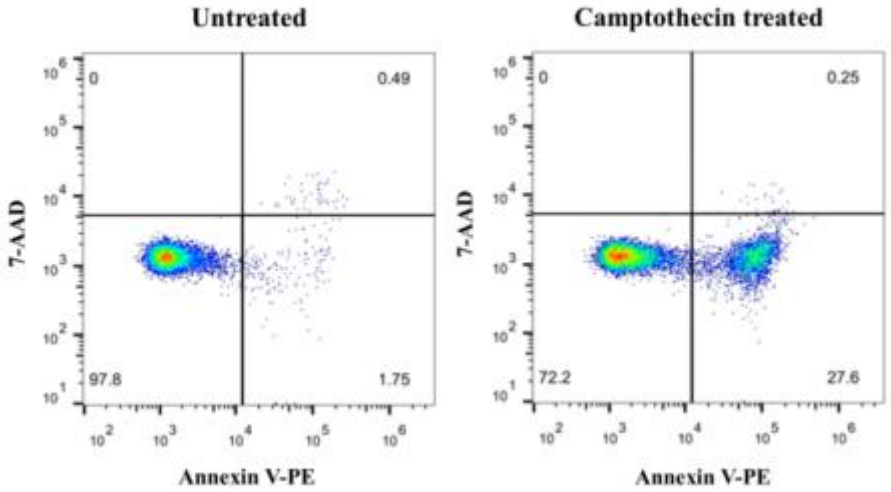
2. Adherent cells: Collect cells culture supernatant first. then digest cells with trypsin without EDTA (recommended G4002 or G4011), combine with the cell culture supernatant and collect the cells by centrifugation at 500 x g for 5 min at 4℃. Trypsin digestion should not be too long to avoid false positives caused by excessive digestion.
3. Wash the cells twice with pre-cooled PBS (recommended G4202), and collect the cells by centrifugation at 500 x g for 5 min at 4℃ each time.
4. Gently resuspend the cells with pre-cooled 1×Binding Buffer, and adjust the cell concentration to 1~5 $\times 10^6$/mL.
5. Add 5 μL of Annexin V-PE and 5 μL of 7-ADD to 100 μL of cell suspension, mix gently and protect from light at room temperature for 8-10 min.
6. Add 400 μL of pre-cooled 1×Binding Buffer, shake gently, and use flow cytometry or fluorescence microscope for detection within 1 hour.

Result Analysis

1. Flow Cytometry Detection

a) Select the appropriate voltage and adjust the light compensation for the flow cytometer analysis. it is recommended to set a negative control (without Annexin V-PE and 7-ADD labeling) to adjust the voltage except for the experimental group, and the single standard control (with Annexin V-PE only, and cells with 7-ADD only) for compensation adjustment.

b) Reference example of flow cytometry detection and analysis: Induce Jurkat T lymphoma cells with 5 μM Camptothecin for 6 h. Referring to the above experimental steps, use flow cytometry to detect. The results are shown in the following figure.



The maximum excitation wavelength of PE is 565 nm and the maximum emission wavelength is 578 nm; the maximum excitation wavelength of 7-ADD-DNA complex is 546 nm and the maximum emission wavelength is 647 nm. A two-colour scatter plot is plotted by flow cytometry correlation analysis software, with PE in the horizontal coordinate and 7-ADD in the vertical coordinate. In a typical experiment, live cells are non-fluorescent and the scatter point is located in the lower left first quadrant. Cells in early apoptosis have a strong orangered fluorescence and the scatter is in the second lower right quadrant. Late stage apoptotic and necrotic cells show dual orangered and pink fluorescence, with the scatter point located in the upper right third quadrant.

2. Fluorescence Microscopy Detection

a) Add 5-10 μL of Annexin V-PE and 7-ADD double-stained cell suspension to the slide.

b) Cover with a coverslip.

c) Observe with a two-color filter under a fluorescence microscope. Annexin V-PE has a orangered fluorescence signal, and 7-ADD has a pink fluorescence signal (When taking pictures with a fluorescence microscope, it is recommended to add an appropriate amount of anti-fluorescence quenching sealer (G1401) to prevent fluorescence quenching problems).

Note

1. The entire experimental process should be handled as gently as possible to avoid cell fragmentation, which may affect the experimental results.
2. Washing the cells with PBS cannot be omitted, and the residual PBS should also be removed as much as possible.
3. When using trypsin to digest cells, the experiment should be handled carefully and the digestion time should be controlled to avoid artificial damage to the cells. If the digestion time is too short, the cells need to be vigorously beaten to fall off, which may easily cause mechanical damage to the cell membrane; if the digestion time is too long, the cell membrane is also easily damaged and the results will be affected. In addition, trypsin containing EDTA cannot be used. EDTA will affect the binding of Annexin V to PS.
4. If some cells are floating after apoptosis stimulation, collect both the cell culture supernatant and the adherent cells to stain for a more accurate result.
5. Annexin V-PE and 7-ADD are sensitive to light, please avoid light during operation. Testing should be performed as soon as possible after the reaction is complete.
6. For your safety and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® Annexin V-IF488/PI Cell Apoptosis Detection Kit

Cat. #: G1513

Product Information

Product Name	Cat. No	Spec.
Annexin V-IF488/PI Cell Apoptosis Detection Kit	G1513-50T	50 T
	G1513-100T	100 T

Product Description/Introduction

Apoptosis is a normal physiological process that occurs during embryonic development and maintenance of tissue homeostasis and is accompanied by many morphological changes, among which the loss of cell membrane is one of the early characteristics of apoptosis. In normal cells, phosphatidylserine (PS) is only distributed on the inner side of the phospholipid bilayer of the cell membrane. However, in the early stage of apoptosis, PS will flip from the inner side of the lipid membrane to the outer side, exposing it to the outside of the cell. Annexin V is a Ca^{2+}-dependent phospholipid-binding protein with high affinity for PS and it can specifically bind to cells exposed to PS. Therefore, Annexin V is used as one of the indicators to detect early apoptosis of cells. Propidium iodide (PI) is a nucleic acid dye that cannot penetrate normal cells with intact cell membranes and early apoptotic cells, but it can penetrate the cell membranes of late apoptotic and necrotic cells and stain cell nuclei.

This product uses a fusion protein composed of IF488 (enhanced Green Fluorescent Protein) and Annexin V as a detection probe to detect early apoptosis of cells. The PI is also used to distinguish live cells from necrotic and late apoptotic cells. In combination with Annexin V-IF488 and PI, live cells show negative staining (Annexin V-/PI-), early apoptotic cells show single fluorescence positive (Annexin V+/PI-), while late apoptotic and necrotic cells show double fluorescence positive (Annexin V+/PI+). This kit is suitable for flow cytometry or fluorescence microscopy detection. It is also suitable for quantitative detection of apoptotic cells as IF488 is fused 1:1 to Annexin V.

Storage and Shipping Conditions

Ship with wet ice; Store at 2-8℃ away from light, valid for 12 months.

Product Components

Component Number	Component	G1513-50T	G1513-100T
G1513-1	Annexin V-IF488	250 μL	2×250 μL
G1513-2	Propidium Iodide (PI)	250 μL	2×250 μL
G1513-3	1×Binding Buffer	25 mL	2×25 mL
Manual		1 pc	

Product Protocol/Procedures

1. Suspension of cells: Take the cell suspension and collect the cells by centrifugation at 500 x g for 5 min at 4℃.
2. Adherent cells: Collect cells culture supernatant first. then digest cells with trypsin without EDTA (recommended G4002 or G4011), combine with the cell culture supernatant and collect the cells by

centrifugation at 500 x g for 5 min at 4℃. Trypsin digestion should not be too long to avoid false positives caused by excessive digestion.

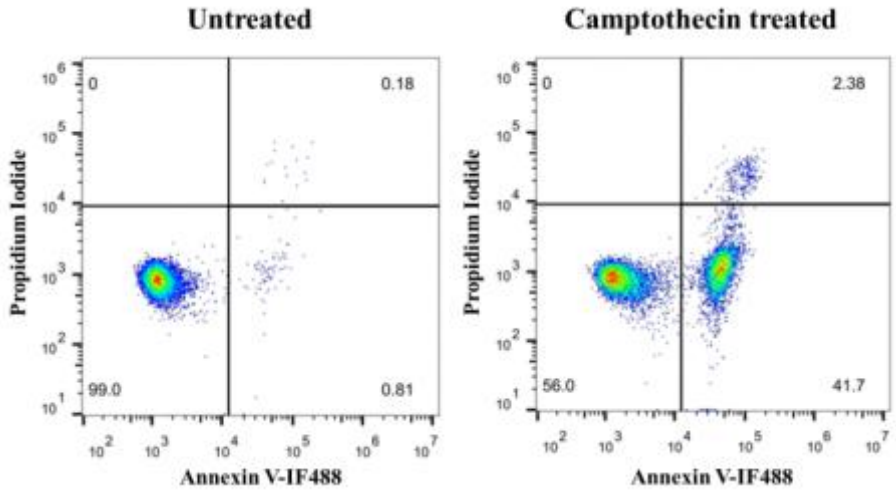
3. Wash the cells twice with pre-cooled PBS (recommended G4202), and collect the cells by centrifugation at 500 x g for 5 min at 4℃ each time.
4. Gently resuspend the cells with pre-cooled 1×Binding Buffer, and adjust the cell concentration to 1~5 $\times 10^6$/mL.
5. Add 5 μL of Annexin V-EGFP and 5 μL of PI to 100 μL of cell suspension, mix gently and protect from light at room temperature for 8-10 min.
6. Add 400 μL of pre-cooled 1×Binding Buffer, shake gently, and use flow cytometry or fluorescence microscope for detection within 1 hour.

Result Analysis

1. Flow Cytometry Detection

a) Select the appropriate voltage and adjust the light compensation for the flow cytometer analysis. it is recommended to set a negative control (without Annexin V-IF488 and PI labeling) to adjust the voltage except for the experimental group, and the single standard control (with Annexin V-IF488 only, and cells with PI only) for compensation adjustment.

b) Reference example of flow cytometry detection and analysis: Induce Jurkat T lymphoma cells with 5 μM Camptothecin for 6 h. Referring to the above experimental steps, use flow cytometry to detect. The results are shown in the following figure.



The maximum excitation wavelength of IF488 is 491 nm and the maximum emission wavelength is 518 nm; the maximum excitation wavelength of PI-DNA complex is 535 nm and the maximum emission wavelength is 615 nm. A two-colour scatter plot is plotted by flow cytometry correlation analysis software, with IF488 in the horizontal coordinate and PI in the vertical coordinate. In a typical experiment, live cells are non-fluorescent and the scatter point is located in the lower left first quadrant. Cells in early apoptosis have a strong green fluorescence and the scatter is in the second lower right quadrant. Late stage apoptotic and necrotic cells show dual red and green fluorescence, with the scatter point located in the upper right third quadrant.

2. Fluorescence Microscopy Detection

a) Add 5-10 μL of Annexin V-IF488 and PI double-stained cell suspension to the slide.

b) Cover with a coverslip.

c) Observe with a two-color filter under a fluorescence microscope. Annexin V-IF488 has a green fluorescence signal, and PI has a red fluorescence signal. (When taking pictures with a fluorescence microscope, it is recommended to add an appropriate amount of anti-fluorescence quenching sealer (G1401) to prevent fluorescence quenching problems)

Note

1. The entire experimental process should be handled as gently as possible to avoid cell fragmentation, which may affect the experimental results.
2. Washing the cells with PBS cannot be omitted, and the residual PBS should also be removed as much as possible.
3. When using trypsin to digest cells, the experiment should be handled carefully and the digestion time should be controlled to avoid artificial damage to the cells. If the digestion time is too short, the cells need to be vigorously beaten to fall off, which may easily cause mechanical damage to the cell membrane; if the digestion time is too long, the cell membrane is also easily damaged and the results will be affected. In addition, trypsin containing EDTA cannot be used. EDTA will affect the binding of Annexin V to PS.
4. If some cells are floating after apoptosis stimulation, collect both the cell culture supernatant and the adherent cells to stain for a more accurate result.
5. Annexin V-IF488 and PI are sensitive to light, please avoid light during operation. Testing should be performed as soon as possible after the reaction is complete.
6. For your safety and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® Annexin V-IF488/PI Cell Apoptosis Detection Kit

Cat. #: G1513

Product Information

Product Name	Cat. No	Spec.
Annexin V-IF488/PI Cell Apoptosis Detection Kit	G1513-50T	50 T
	G1513-100T	100 T

Product Description/Introduction

Apoptosis is a normal physiological process that occurs during embryonic development and maintenance of tissue homeostasis and is accompanied by many morphological changes, among which the loss of cell membrane is one of the early characteristics of apoptosis. In normal cells, phosphatidylserine (PS) is only distributed on the inner side of the phospholipid bilayer of the cell membrane. However, in the early stage of apoptosis, PS will flip from the inner side of the lipid membrane to the outer side, exposing it to the outside of the cell. Annexin V is a Ca^{2+}-dependent phospholipid-binding protein with high affinity for PS and it can specifically bind to cells exposed to PS. Therefore, Annexin V is used as one of the indicators to detect early apoptosis of cells. Propidium iodide (PI) is a nucleic acid dye that cannot penetrate normal cells with intact cell membranes and early apoptotic cells, but it can penetrate the cell membranes of late apoptotic and necrotic cells and stain cell nuclei.

This product uses a fusion protein composed of IF488 (enhanced Green Fluorescent Protein) and Annexin V as a detection probe to detect early apoptosis of cells. The PI is also used to distinguish live cells from necrotic and late apoptotic cells. In combination with Annexin V-IF488 and PI, live cells show negative staining (Annexin V-/PI-), early apoptotic cells show single fluorescence positive (Annexin V+/PI-), while late apoptotic and necrotic cells show double fluorescence positive (Annexin V+/PI+). This kit is suitable for flow cytometry or fluorescence microscopy detection. It is also suitable for quantitative detection of apoptotic cells as IF488 is fused 1:1 to Annexin V.

Storage and Shipping Conditions

Ship with wet ice; Store at 2-8℃ away from light, valid for 12 months.

Product Components

Component Number	Component	G1513-50T	G1513-100T
G1513-1	Annexin V-IF488	250 μL	2×250 μL
G1513-2	Propidium Iodide (PI)	250 μL	2×250 μL
G1513-3	1×Binding Buffer	25 mL	2×25 mL
Manual		1 pc	

Product Protocol/Procedures

1. Suspension of cells: Take the cell suspension and collect the cells by centrifugation at 500 x g for 5 min at 4℃.
2. Adherent cells: Collect cells culture supernatant first. then digest cells with trypsin without EDTA (recommended G4002 or G4011), combine with the cell culture supernatant and collect the cells by

centrifugation at 500 x g for 5 min at 4℃. Trypsin digestion should not be too long to avoid false positives caused by excessive digestion.

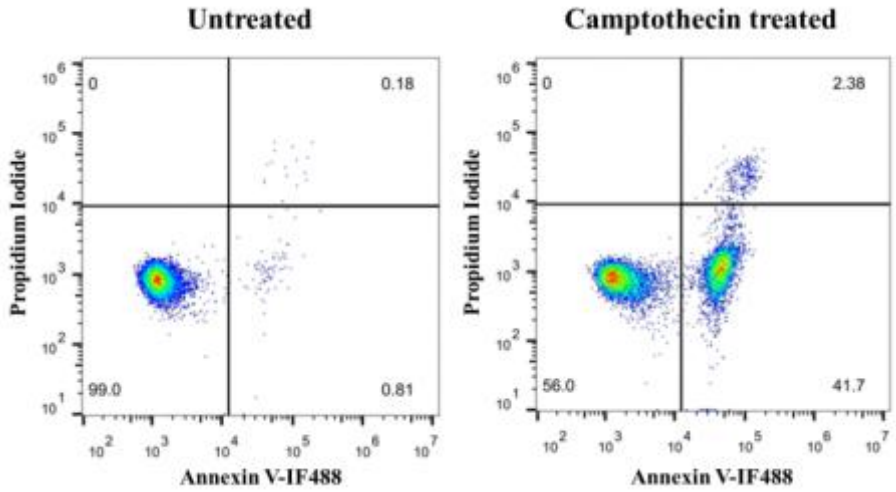
3. Wash the cells twice with pre-cooled PBS (recommended G4202), and collect the cells by centrifugation at 500 x g for 5 min at 4℃ each time.
4. Gently resuspend the cells with pre-cooled 1×Binding Buffer, and adjust the cell concentration to 1~5 $\times 10^6$/mL.
5. Add 5 μL of Annexin V-EGFP and 5 μL of PI to 100 μL of cell suspension, mix gently and protect from light at room temperature for 8-10 min.
6. Add 400 μL of pre-cooled 1×Binding Buffer, shake gently, and use flow cytometry or fluorescence microscope for detection within 1 hour.

Result Analysis

1. Flow Cytometry Detection

a) Select the appropriate voltage and adjust the light compensation for the flow cytometer analysis. it is recommended to set a negative control (without Annexin V-IF488 and PI labeling) to adjust the voltage except for the experimental group, and the single standard control (with Annexin V-IF488 only, and cells with PI only) for compensation adjustment.

b) Reference example of flow cytometry detection and analysis: Induce Jurkat T lymphoma cells with 5 μM Camptothecin for 6 h. Referring to the above experimental steps, use flow cytometry to detect. The results are shown in the following figure.



The maximum excitation wavelength of IF488 is 491 nm and the maximum emission wavelength is 518 nm; the maximum excitation wavelength of PI-DNA complex is 535 nm and the maximum emission wavelength is 615 nm. A two-colour scatter plot is plotted by flow cytometry correlation analysis software, with IF488 in the horizontal coordinate and PI in the vertical coordinate. In a typical experiment, live cells are non-fluorescent and the scatter point is located in the lower left first quadrant. Cells in early apoptosis have a strong green fluorescence and the scatter is in the second lower right quadrant. Late stage apoptotic and necrotic cells show dual red and green fluorescence, with the scatter point located in the upper right third quadrant.

2. Fluorescence Microscopy Detection

a) Add 5-10 μL of Annexin V-IF488 and PI double-stained cell suspension to the slide.

b) Cover with a coverslip.

c) Observe with a two-color filter under a fluorescence microscope. Annexin V-IF488 has a green fluorescence signal, and PI has a red fluorescence signal. (When taking pictures with a fluorescence microscope, it is recommended to add an appropriate amount of anti-fluorescence quenching sealer (G1401) to prevent fluorescence quenching problems)

Note

1. The entire experimental process should be handled as gently as possible to avoid cell fragmentation, which may affect the experimental results.
2. Washing the cells with PBS cannot be omitted, and the residual PBS should also be removed as much as possible.
3. When using trypsin to digest cells, the experiment should be handled carefully and the digestion time should be controlled to avoid artificial damage to the cells. If the digestion time is too short, the cells need to be vigorously beaten to fall off, which may easily cause mechanical damage to the cell membrane; if the digestion time is too long, the cell membrane is also easily damaged and the results will be affected. In addition, trypsin containing EDTA cannot be used. EDTA will affect the binding of Annexin V to PS.
4. If some cells are floating after apoptosis stimulation, collect both the cell culture supernatant and the adherent cells to stain for a more accurate result.
5. Annexin V-IF488 and PI are sensitive to light, please avoid light during operation. Testing should be performed as soon as possible after the reaction is complete.
6. For your safety and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® Annexin V-IF647/PI Cell Apoptosis Detection Kit

Cat. #: G1514

Product Information

Product Name	Cat. No	Spec.
Annexin V-IF647/PI Cell Apoptosis Detection Kit	G1514-50T	50 T
	G1514-100T	100 T

Product Description/Introduction

Apoptosis is a normal physiological process that occurs during embryonic development and maintenance of tissue homeostasis and is accompanied by many morphological changes, among which the loss of cell membrane is one of the early characteristics of apoptosis. In normal cells, phosphatidylserine (PS) is only distributed on the inner side of the phospholipid bilayer of the cell membrane. However, in the early stage of apoptosis, PS will flip from the inner side of the lipid membrane to the outer side, exposing it to the outside of the cell. Annexin V is a Ca^{2+}-dependent phospholipid-binding protein with high affinity for PS and it can specifically bind to cells exposed to PS. Therefore, Annexin V is used as one of the indicators to detect early apoptosis of cells. Propidium iodide (PI) is a nucleic acid dye that cannot penetrate normal cells with intact cell membranes and early apoptotic cells, but it can penetrate the cell membranes of late apoptotic and necrotic cells and stain cell nuclei.

This product uses a fusion protein composed of IF647 (enhanced Green Fluorescent Protein) and Annexin V as a detection probe to detect early apoptosis of cells. The PI is also used to distinguish live cells from necrotic and late apoptotic cells. In combination with Annexin V-IF647 and PI, live cells show negative staining (Annexin V-/PI-), early apoptotic cells show single fluorescence positive (Annexin V+/PI-), while late apoptotic and necrotic cells show double fluorescence positive (Annexin V+/PI+). This kit is suitable for flow cytometry or fluorescence microscopy detection. It is also suitable for quantitative detection of apoptotic cells as IF647 is fused 1:1 to Annexin V.

Storage and Shipping Conditions

Ship with wet ice; Store at 2-8℃ away from light, valid for 12 months.

Product Components

Component Number	Component	G1514-50T	G1514-100T
G1514-1	Annexin V-IF647	250 μL	2×250 μL
G1514-2	Propidium Iodide (PI)	250 μL	2×250 μL
G1514-3	1×Binding Buffer	25 mL	2×25 mL
Manual		1 pc	

Product Protocol/Procedures

1. Suspension of cells: Take the cell suspension and collect the cells by centrifugation at 500 x g for 5 min at 4℃;

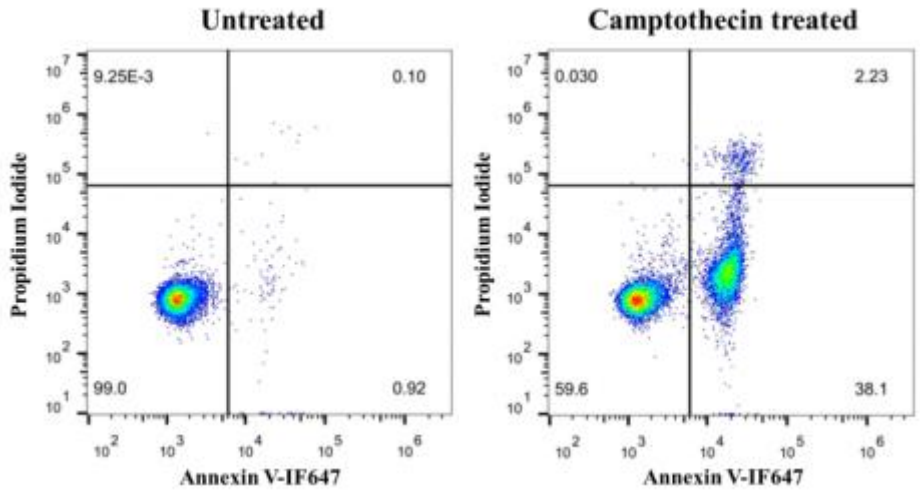
2. Adherent cells: Collect cells culture supernatant first. then digest cells with trypsin without EDTA (recommended G4002 or G4011), combine with the cell culture supernatant and collect the cells by centrifugation at 500 x g for 5 min at 4℃. Trypsin digestion should not be too long to avoid false positives caused by excessive digestion.
3. Wash the cells twice with pre-cooled PBS (recommended G4202), and collect the cells by centrifugation at 500 x g for 5 min at 4℃ each time;
4. Gently resuspend the cells with pre-cooled 1×Binding Buffer, and adjust the cell concentration to 1~5 $\times 10^6$/mL;
5. Add 5 μL of Annexin V-EGFP and 5 μL of PI to 100 μL of cell suspension, mix gently and protect from light at room temperature for 8-10 min.
6. Add 400 μL of pre-cooled 1×Binding Buffer, shake gently, and use flow cytometry or fluorescence microscope for detection within 1 hour.

Result Analysis

1. Flow Cytometry Detection

a) Select the appropriate voltage and adjust the light compensation for the flow cytometer analysis. it is recommended to set a negative control (without Annexin V-IF647 and PI labeling) to adjust the voltage except for the experimental group, and the single standard control (with Annexin V-IF647 only, and cells with PI only) for compensation adjustment.

b) Reference example of flow cytometry detection and analysis: Induce Jurkat T lymphoma cells with 5 μM Camptothecin for 6 h. Referring to the above experimental steps, use flow cytometry to detect. The results are shown in the following figure.



The maximum excitation wavelength of IF647 is 656 nm and the maximum emission wavelength is 670 nm; the maximum excitation wavelength of PI-DNA complex is 535 nm and the maximum emission wavelength is 615 nm. A two-colour scatter plot is plotted by flow cytometry correlation analysis software, with IF647 in the horizontal coordinate and PI in the vertical coordinate. In a typical experiment, live cells are non-fluorescent and the scatter point is located in the lower left first quadrant. Cells in early apoptosis have a strong green fluorescence and the scatter is in the second lower right quadrant. Late stage apoptotic and necrotic cells show dual red and green fluorescence, with the scatter point located in the upper right third quadrant.

2. Fluorescence Microscopy Detection

a) Add 5-10 μL of Annexin V-IF647 and PI double-stained cell suspension to the slide.

b) Cover with a coverslip.

c) Observe with a two-color filter under a fluorescence microscope. Annexin V-IF647 has a green fluorescence signal, and PI has a red fluorescence signal.(When taking pictures with a fluorescence microscope, it is recommended to add an appropriate amount of anti-fluorescence quenching sealer (G1401) to prevent fluorescence quenching problems)

Note

1. The entire experimental process should be handled as gently as possible to avoid cell fragmentation, which may affect the experimental results.
2. Washing the cells with PBS cannot be omitted, and the residual PBS should also be removed as much as possible.
3. When using trypsin to digest cells, the experiment should be handled carefully and the digestion time should be controlled to avoid artificial damage to the cells. If the digestion time is too short, the cells need to be vigorously beaten to fall off, which may easily cause mechanical damage to the cell membrane; if the digestion time is too long, the cell membrane is also easily damaged and the results will be affected. In addition, trypsin containing EDTA cannot be used. EDTA will affect the binding of Annexin V to PS.
4. If some cells are floating after apoptosis stimulation, collect both the cell culture supernatant and the adherent cells to stain for a more accurate result.
5. Annexin V-IF647 and PI are sensitive to light, please avoid light during operation. Testing should be performed as soon as possible after the reaction is complete.
6. For your safety and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® Annexin V-IF647/PI Cell Apoptosis Detection Kit

Cat. #: G1514

Product Information

Product Name	Cat. No	Spec.
Annexin V-IF647/PI Cell Apoptosis Detection Kit	G1514-50T	50 T
	G1514-100T	100 T

Product Description/Introduction

Apoptosis is a normal physiological process that occurs during embryonic development and maintenance of tissue homeostasis and is accompanied by many morphological changes, among which the loss of cell membrane is one of the early characteristics of apoptosis. In normal cells, phosphatidylserine (PS) is only distributed on the inner side of the phospholipid bilayer of the cell membrane. However, in the early stage of apoptosis, PS will flip from the inner side of the lipid membrane to the outer side, exposing it to the outside of the cell. Annexin V is a Ca^{2+}-dependent phospholipid-binding protein with high affinity for PS and it can specifically bind to cells exposed to PS. Therefore, Annexin V is used as one of the indicators to detect early apoptosis of cells. Propidium iodide (PI) is a nucleic acid dye that cannot penetrate normal cells with intact cell membranes and early apoptotic cells, but it can penetrate the cell membranes of late apoptotic and necrotic cells and stain cell nuclei.

This product uses a fusion protein composed of IF647 (enhanced Green Fluorescent Protein) and Annexin V as a detection probe to detect early apoptosis of cells. The PI is also used to distinguish live cells from necrotic and late apoptotic cells. In combination with Annexin V-IF647 and PI, live cells show negative staining (Annexin V-/PI-), early apoptotic cells show single fluorescence positive (Annexin V+/PI-), while late apoptotic and necrotic cells show double fluorescence positive (Annexin V+/PI+). This kit is suitable for flow cytometry or fluorescence microscopy detection. It is also suitable for quantitative detection of apoptotic cells as IF647 is fused 1:1 to Annexin V.

Storage and Shipping Conditions

Ship with wet ice; Store at 2-8℃ away from light, valid for 12 months.

Product Components

Component Number	Component	G1514-50T	G1514-100T
G1514-1	Annexin V-IF647	250 μL	2×250 μL
G1514-2	Propidium Iodide (PI)	250 μL	2×250 μL
G1514-3	1×Binding Buffer	25 mL	2×25 mL
Manual		1 pc	

Product Protocol/Procedures

1. Suspension of cells: Take the cell suspension and collect the cells by centrifugation at 500 x g for 5 min at 4℃;

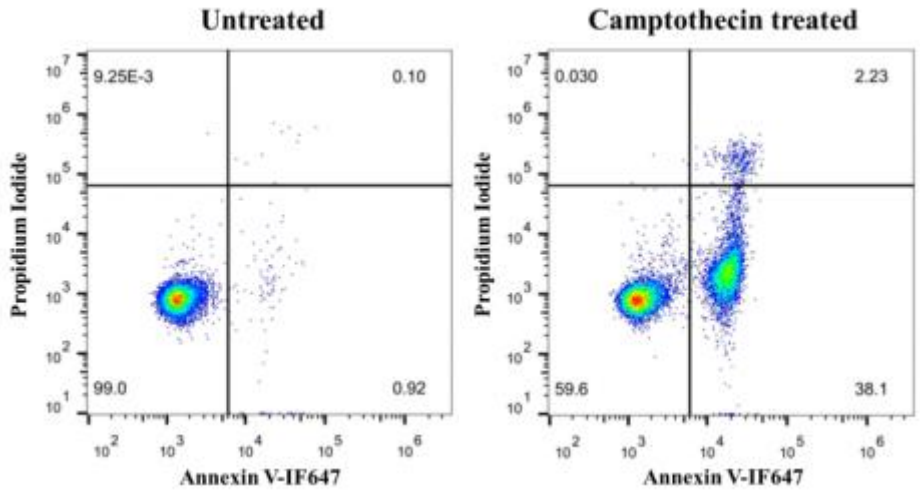
2. Adherent cells: Collect cells culture supernatant first. then digest cells with trypsin without EDTA (recommended G4002 or G4011), combine with the cell culture supernatant and collect the cells by centrifugation at 500 x g for 5 min at 4℃. Trypsin digestion should not be too long to avoid false positives caused by excessive digestion.
3. Wash the cells twice with pre-cooled PBS (recommended G4202), and collect the cells by centrifugation at 500 x g for 5 min at 4℃ each time;
4. Gently resuspend the cells with pre-cooled 1×Binding Buffer, and adjust the cell concentration to 1~5 $\times 10^6$/mL;
5. Add 5 μL of Annexin V-EGFP and 5 μL of PI to 100 μL of cell suspension, mix gently and protect from light at room temperature for 8-10 min.
6. Add 400 μL of pre-cooled 1×Binding Buffer, shake gently, and use flow cytometry or fluorescence microscope for detection within 1 hour.

Result Analysis

1. Flow Cytometry Detection

a) Select the appropriate voltage and adjust the light compensation for the flow cytometer analysis. it is recommended to set a negative control (without Annexin V-IF647 and PI labeling) to adjust the voltage except for the experimental group, and the single standard control (with Annexin V-IF647 only, and cells with PI only) for compensation adjustment.

b) Reference example of flow cytometry detection and analysis: Induce Jurkat T lymphoma cells with 5 μM Camptothecin for 6 h. Referring to the above experimental steps, use flow cytometry to detect. The results are shown in the following figure.



The maximum excitation wavelength of IF647 is 656 nm and the maximum emission wavelength is 670 nm; the maximum excitation wavelength of PI-DNA complex is 535 nm and the maximum emission wavelength is 615 nm. A two-colour scatter plot is plotted by flow cytometry correlation analysis software, with IF647 in the horizontal coordinate and PI in the vertical coordinate. In a typical experiment, live cells are non-fluorescent and the scatter point is located in the lower left first quadrant. Cells in early apoptosis have a strong green fluorescence and the scatter is in the second lower right quadrant. Late stage apoptotic and necrotic cells show dual red and green fluorescence, with the scatter point located in the upper right third quadrant.

2. Fluorescence Microscopy Detection

a) Add 5-10 μL of Annexin V-IF647 and PI double-stained cell suspension to the slide.

b) Cover with a coverslip.

c) Observe with a two-color filter under a fluorescence microscope. Annexin V-IF647 has a green fluorescence signal, and PI has a red fluorescence signal.(When taking pictures with a fluorescence microscope, it is recommended to add an appropriate amount of anti-fluorescence quenching sealer (G1401) to prevent fluorescence quenching problems)

Note

1. The entire experimental process should be handled as gently as possible to avoid cell fragmentation, which may affect the experimental results.
2. Washing the cells with PBS cannot be omitted, and the residual PBS should also be removed as much as possible.
3. When using trypsin to digest cells, the experiment should be handled carefully and the digestion time should be controlled to avoid artificial damage to the cells. If the digestion time is too short, the cells need to be vigorously beaten to fall off, which may easily cause mechanical damage to the cell membrane; if the digestion time is too long, the cell membrane is also easily damaged and the results will be affected. In addition, trypsin containing EDTA cannot be used. EDTA will affect the binding of Annexin V to PS.
4. If some cells are floating after apoptosis stimulation, collect both the cell culture supernatant and the adherent cells to stain for a more accurate result.
5. Annexin V-IF647 and PI are sensitive to light, please avoid light during operation. Testing should be performed as soon as possible after the reaction is complete.
6. For your safety and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® Annexin V-IF647/PI Cell Apoptosis Detection Kit

Cat. #: G1514

Product Information

Product Name	Cat. No	Spec.
Annexin V-IF647/PI Cell Apoptosis Detection Kit	G1514-50T	50 T
	G1514-100T	100 T

Product Description/Introduction

Apoptosis is a normal physiological process that occurs during embryonic development and maintenance of tissue homeostasis and is accompanied by many morphological changes, among which the loss of cell membrane is one of the early characteristics of apoptosis. In normal cells, phosphatidylserine (PS) is only distributed on the inner side of the phospholipid bilayer of the cell membrane. However, in the early stage of apoptosis, PS will flip from the inner side of the lipid membrane to the outer side, exposing it to the outside of the cell. Annexin V is a Ca^{2+}-dependent phospholipid-binding protein with high affinity for PS and it can specifically bind to cells exposed to PS. Therefore, Annexin V is used as one of the indicators to detect early apoptosis of cells. Propidium iodide (PI) is a nucleic acid dye that cannot penetrate normal cells with intact cell membranes and early apoptotic cells, but it can penetrate the cell membranes of late apoptotic and necrotic cells and stain cell nuclei.

This product uses a fusion protein composed of IF647 (enhanced Green Fluorescent Protein) and Annexin V as a detection probe to detect early apoptosis of cells. The PI is also used to distinguish live cells from necrotic and late apoptotic cells. In combination with Annexin V-IF647 and PI, live cells show negative staining (Annexin V-/PI-), early apoptotic cells show single fluorescence positive (Annexin V+/PI-), while late apoptotic and necrotic cells show double fluorescence positive (Annexin V+/PI+). This kit is suitable for flow cytometry or fluorescence microscopy detection. It is also suitable for quantitative detection of apoptotic cells as IF647 is fused 1:1 to Annexin V.

Storage and Shipping Conditions

Ship with wet ice; Store at 2-8℃ away from light, valid for 12 months.

Product Components

Component Number	Component	G1514-50T	G1514-100T
G1514-1	Annexin V-IF647	250 μL	2×250 μL
G1514-2	Propidium Iodide (PI)	250 μL	2×250 μL
G1514-3	1×Binding Buffer	25 mL	2×25 mL
Manual		1 pc	

Product Protocol/Procedures

1. Suspension of cells: Take the cell suspension and collect the cells by centrifugation at 500 x g for 5 min at 4℃;

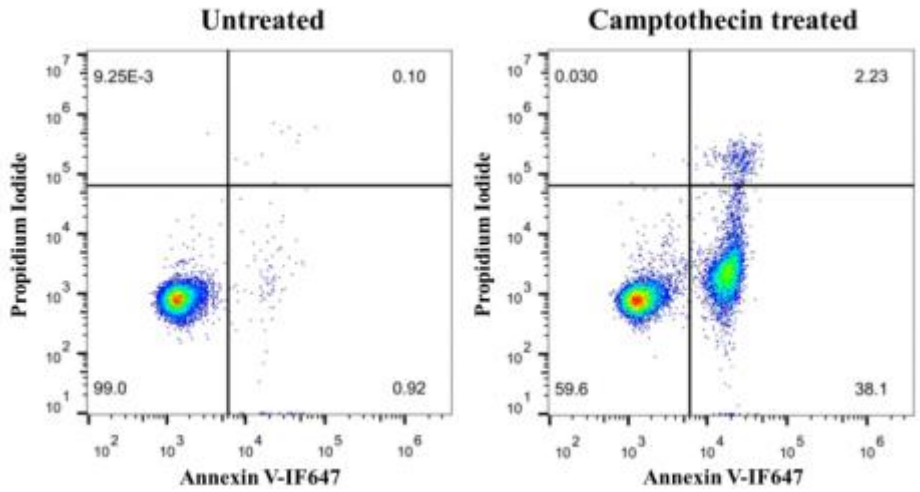
2. Adherent cells: Collect cells culture supernatant first. then digest cells with trypsin without EDTA (recommended G4002 or G4011), combine with the cell culture supernatant and collect the cells by centrifugation at 500 x g for 5 min at 4℃. Trypsin digestion should not be too long to avoid false positives caused by excessive digestion.
3. Wash the cells twice with pre-cooled PBS (recommended G4202), and collect the cells by centrifugation at 500 x g for 5 min at 4℃ each time;
4. Gently resuspend the cells with pre-cooled 1×Binding Buffer, and adjust the cell concentration to 1~5 $\times 10^6$/mL;
5. Add 5 μL of Annexin V-EGFP and 5 μL of PI to 100 μL of cell suspension, mix gently and protect from light at room temperature for 8-10 min.
6. Add 400 μL of pre-cooled 1×Binding Buffer, shake gently, and use flow cytometry or fluorescence microscope for detection within 1 hour.

Result Analysis

1. Flow Cytometry Detection

a) Select the appropriate voltage and adjust the light compensation for the flow cytometer analysis. it is recommended to set a negative control (without Annexin V-IF647 and PI labeling) to adjust the voltage except for the experimental group, and the single standard control (with Annexin V-IF647 only, and cells with PI only) for compensation adjustment.

b) Reference example of flow cytometry detection and analysis: Induce Jurkat T lymphoma cells with 5 μM Camptothecin for 6 h. Referring to the above experimental steps, use flow cytometry to detect. The results are shown in the following figure.



The maximum excitation wavelength of IF647 is 656 nm and the maximum emission wavelength is 670 nm; the maximum excitation wavelength of PI-DNA complex is 535 nm and the maximum emission wavelength is 615 nm. A two-colour scatter plot is plotted by flow cytometry correlation analysis software, with IF647 in the horizontal coordinate and PI in the vertical coordinate. In a typical experiment, live cells are non-fluorescent and the scatter point is located in the lower left first quadrant. Cells in early apoptosis have a strong green fluorescence and the scatter is in the second lower right quadrant. Late stage apoptotic and necrotic cells show dual red and green fluorescence, with the scatter point located in the upper right third quadrant.

2. Fluorescence Microscopy Detection

a) Add 5-10 μL of Annexin V-IF647 and PI double-stained cell suspension to the slide.

b) Cover with a coverslip.

c) Observe with a two-color filter under a fluorescence microscope. Annexin V-IF647 has a green fluorescence signal, and PI has a red fluorescence signal.(When taking pictures with a fluorescence microscope, it is recommended to add an appropriate amount of anti-fluorescence quenching sealer (G1401) to prevent fluorescence quenching problems)

Note

1. The entire experimental process should be handled as gently as possible to avoid cell fragmentation, which may affect the experimental results.
2. Washing the cells with PBS cannot be omitted, and the residual PBS should also be removed as much as possible.
3. When using trypsin to digest cells, the experiment should be handled carefully and the digestion time should be controlled to avoid artificial damage to the cells. If the digestion time is too short, the cells need to be vigorously beaten to fall off, which may easily cause mechanical damage to the cell membrane; if the digestion time is too long, the cell membrane is also easily damaged and the results will be affected. In addition, trypsin containing EDTA cannot be used. EDTA will affect the binding of Annexin V to PS.
4. If some cells are floating after apoptosis stimulation, collect both the cell culture supernatant and the adherent cells to stain for a more accurate result.
5. Annexin V-IF647 and PI are sensitive to light, please avoid light during operation. Testing should be performed as soon as possible after the reaction is complete.
6. For your safety and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® Click-iT 594 Tunel Cell Apoptosis Detection Kit

Cat. No.: G1516-50T

Product Information

Product Name	Cat. No.	Spec.
Click-iT 594 Tunel Cell Apoptosis Detection Kit	G1516-50T	50T

Product Description/Introduction

The breaking of chromosomal DNA in apoptosis is a gradual, phased process. Chromosomal DNA is first degraded into large 50-300 kb segments by endogenous nucleic acid hydrolases, and then about 30% of chromosomal DNA is randomly cut between nucleosome units in the presence of Ca^{2+}- and Mg^{2+}-dependent nucleic acid endonucleases to form 180-200 bp nucleosomal DNA multimers.Thus, in late apoptosis, DNA is degraded into 180-200 bp fragments, and a large number of 3'-OH ends are exposed on the broken genomic DNA. Terminal Deoxynucleotidyl Transferase (TdT) is a template-independent DNA polymerase that catalyzes the incorporation of deoxyribonucleotides into the 3'-OH ends of broken DNA molecules. Therefore TUNEL (TdT mediated dUTP Nick End Labeling) Cell Apoptosis Detection Kit can be used to detect the breakage of nuclear DNA in tissue cells during late apoptosis.

The principle is that under the action of TdT enzyme, EdUTP (a dUTP with alkyne modification) is doped into the 3′-OH end exposed during genomic DNA breakage, and the alkyne group reacts with the azide dye in a ring-forming reaction catalyzed by a monovalent copper ion (click reaction) thus introducing the fluorescent moiety in a targeted way. iF594 azide is used in this kit, thus it can be detected by fluorescence microscope or flow cytometry (iF594 excitation 593 nm, emission 614 nm). Compared to other modified dUTPs, EdUTP has a smaller spatial site resistance and is more easily doped into DNA ends by TdTase.

This kit has a wide range of applications and is suitable for apoptosis detection in paraffin tissue sections, frozen tissue sections, cell crawls and cell smears.

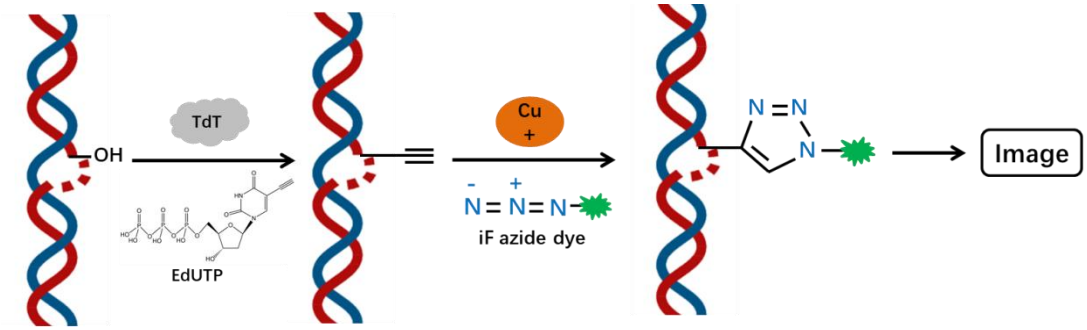


Figure 1. Schematic diagram of the click chemistry-based TUNEL kit principle

Storage and Shipping Conditions

Ship with wet ice; Store at -20℃,valid for 12 months.

Product Content

Component Number	Component	G1516-50T
G1516-1	Recombinant Tdt Enzyme	50 μL
G1516-2	EdUTP Labeling Mix	250 μL
G1516-3	Equilibration Buffer	5×1 mL

G1516-4	Proteinase K (200 μg/mL)	1 mL
G1516-5	iF594 azide dye	80 μL
G1516-6	Reaction Buffer A	5×1 mL
G1516-7	Reaction Buffer B	60 μL
G1516-8	Reaction additive (Reagent C)	2×100 mg
Manual		1 pc

Pre-experimentation

1. PBS phosphate buffer (recommended G0002 or G4202);
2. Fixed solution: 4% paraformaldehyde dissolved in PBS, pH 7.4 (recommended G1101);
3. Membrane-breaking solution: 0.1% Triton X-100 dissolved in 0.1% sodium citrate (recommended G1204);
4. 0.2% Triton X-100 prepared in PBS; 0.1% Triton X-100 prepared in PBS containing 5 mg/mL BSA;
5. For nuclear staining, provide your own DAPI (2 μg/mL), Hoechst 33258, or PI (1 μg/mL) (G1012, G1011, G1021 recommended);
6. DNase I (G3342) for positive control experiments;
7. Reaction additive (Reagent C) is centrifuged at low speed, 100 mg of the powder is dissolved in 1 mL of ultrapure water (ready to use), and 100 μL is dispensed and stored at -20°C, leaving the remaining powder for spare use; (Reagent C is easy to oxidize, try to avoid long time exposure to the air, after formulated into an aqueous solution, it is strongly recommended to divide into small portions for use; After testing the aqueous solution of Reagent C, the color of a slight change, click the reaction system can still be carried out normally, and if it shows brown color, it indicates that the component has been invalidated)
8. If the background color of the result is too dark, it may be caused by insufficient washing and residual fixative during the experiment;
9. Be careful to add the reaction liquid in sequence, and mix well while adding;
10. For your health and safety, please wear lab coat and gloves during operation.

Assay Protocol / Procedures

1. Sample Preparation

A. Paraffin-embedded tissue sections

a. The paraffin tissue sections are soaked in xylene for 5-10 min at room temperature and repeated 3 times; Then soak in anhydrous ethanol for 5 min and repeat twice; Finally, soak in gradient ethanol (85%, 75%, and double-distilled water) once for 5 min each;

b. Gently moisten the section with PBS and remove excess liquid around the sample, use a PAP Pen to draw a small circle spaced 2-3 mm apart from the tissue along the tissue's peripheral contour to facilitate downstream permeability processing and equilibrium labeling operations, do not allow the sample to dry out during experiments, and keep the processed samples in a wet box to keep the samples moist;

c. **Preparation of Proteinase K working solution**: Dilute the Proteinase K (200 μg/mL) stock solution with PBS as diluent at a ratio of 1:9 to give a final concentration of 20 μg/mL;

d. Add 100 μL of the above Proteinase K working solution to each sample to make it fully covered, and incubate at 37°C for 20 min;

Note: Proteinase K treatment mainly contributes to the permeation of staining reagents in the subsequent steps of tissues and cells, and its incubation time is too long or too short to affect the efficiency of the subsequent labeling, in order to get better results, the incubation time can be

optimized according to the actual situation.

e. Wash the samples by moistening with PBS solution 3 times for 5 min each time (Proteinase K needs to be washed clean or it will interfere with the subsequent labeling reaction). The treated samples were placed in a wet box to keep the samples moist;

f. (Optional step) Remove the excess liquid on the sample, add appropriate amount of membrane-breaking solution droplets to the tissue, fully infiltrate the tissue, and treat it for 20 min at room temperature; after the membrane-breaking treatment is completed, the sample is similarly washed with PBS solution for 3 times, each time for 5 min; the treated sample is placed in a wet box to keep the sample moist.

B. Tissue frozen section

a. The tissue frozen sections are submerged in the fixative and incubated and fixed for 10-15 min at room temperature;

b. The tissue sections are removed from the fixative and placed in a fume hood to dry naturally;

c. Tissue sections are moistened and washed in purified water or PBS to remove residual fixative from the sample;

d. Use a PAP Pen to draw a small circle spaced 2-3 mm apart from the tissue along the tissue's peripheral contour to facilitate downstream permeability processing and equilibrium labeling operations, do not allow the sample to dry out during experiments, and keep the processed samples in a wet box to keep the samples moist;

e. Preparation of Proteinase K working solution: Dilute the Proteinase K (200 μg/mL) stock solution with PBS as diluent at a ratio of 1:9 to give a final concentration of 20 μg/mL;

f. Add 100 μL of the above Proteinase K working solution to each sample to make it fully covered, and incubate for 10 min at room temperature;

Note: Proteinase K treatment mainly contributes to the permeation of staining reagents in the subsequent steps of tissues and cells, and its incubation time is too long or too short to affect the efficiency of the subsequent labeling, in order to get better results, the incubation time can be optimized according to the actual situation.

g. Wash the sample 2-3 times with PBS solution to remove excess liquid (Proteinase K needs to be washed clean or it will interfere with the subsequent labeling reaction), and keep the treated sample in a wet box to keep the sample moist;

h. (Optional step) Add appropriate amount of membrane-breaking solution droplets to the tissue, fully infiltrate the tissue, room temperature treatment for 20 min, membrane-breaking treatment is completed the same after the completion of the sample with the PBS solution to rinse, to remove the excess liquid, after the treatment of the sample in the wet box to keep the sample wet.

C. Cell crawler

a. Adherent cells are cultured on Lab-Tek slide chambers (Chamber Slides), and slides are gently moistened and washed 2 times with PBS after apoptosis induction treatment;

b. Appropriate amount of fixative is added to each slide chamber to cover the tissue and incubated for 20 min at room temperature;

c. Remove the fixative and add PBS to wash 3 times for 5 min each;

d. Each sample is immersed in 0.2% Triton X-100 solution prepared in PBS and incubated for 5 min at room temperature for permeabilization;

e. Wash samples by submerging them 2-3 times in an open beaker containing PBS solution;

f. Gently remove excess liquid and use filter paper to carefully blot the liquid around the sample on the slide.The processed sample is placed in a wet box to keep the sample moist.

D. Cell smear

a. Cells are resuspended in PBS at a concentration of about 2 × 10^7 cells/mL, and 50-100 μL of cell suspension is aspirated onto an anti-detachment slide, and a clean slide is used to gently spread the cell suspension;

b. The cell smears are immersed in a staining jar filled with fixing solution, and the cells are fixed and left at 4°C for 25 min;

c. Immerse the slide in PBS and leave it at room temperature for 5 min to soak and rinse, repeat once;

d. Gently remove the excess liquid and carefully blot the excess liquid around the sample on the slide with filter paper, draw a small circle along the peripheral contour of the cell with a PAP Pen to facilitate downstream permeability processing and equilibrium labeling operations, and do not allow the sample to dry out during the course of the experiment;

e. Each sample is immersed in 0.2% Triton X-100 solution prepared in PBS and incubated for 5 min at room temperature for permeabilization;

f. Wash samples by submerging them 2-3 times in an open beaker containing PBS solutio

g. Gently remove excess liquid and carefully blot the liquid around the sample on the slide with filter paper. Keep the sample moist by placing the treated sample in a wet box.

2. DNase I treatment positive control experiment (optional step)

After sample permeabilization treatment, samples are treated with DNase I (recommended G3342) to prepare positive controls.

2.1. 100 μL of 1×DNase I Buffer (preparation: take 10 μL of 10×DNase I Buffer, then add 90 μL of deionized water and mix well) is added dropwise to the permeabilized samples and incubated for 5 min at room temperature;

2.2. Gently remove the excess liquid, add 100 μL of working solution containing DNase I (20 U/mL) (preparation method: take 10 μL of 10× DNase I Buffer, then add 2 μL of DNase I, and then add 88 μL of deionized water to mix), and incubate for 10 min at room temperature;

2.3. Gently remove excess liquid and wash the slides thoroughly 3-4 times in a staining vat filled with PBS.

Note: Positive control slides must be stained using a separate staining vat, otherwise residual DNase I on the positive control slides may introduce a high background on the experimental slides.

3. Marking and detection

3.1. **Equilibration:** Add 50 μL Equilibration Buffer to each sample to cover all the sample area and incubate for 10 min at room temperature;

3.2. **Labeling solution preparation**: Thaw EdUTP Labeling Mix and Equilibration Buffer on ice and mix enough TdT incubation buffer for all experiments according to the ratio of Recombinant TdT enzyme: EdUTP Labeling Mix: Equilibration Buffer=1 μL:5 μL:50 μL (1:5:50), the volume of reagents used in the specific experiments can be adjusted in equal proportions according to the size of the slides;

3.3. **Negative control system**: Prepare a control TdT incubation buffer without Recombinant TdT enzyme and replace it with ddH2O;

3.4. **Labeling**: Remove as much Equilibration Buffer as possible, then add 56 μL of TdT Incubation Buffer to each tissue sample and incubate at 37°C for 1 h; be careful not to dry the slides;

3.5. Wash the tissue samples immediately with PBS for 4 washes of 5 min each;

3.6. **Click reaction**: Remove the PBS buffer from the previous step, add 100 μL of click reaction solution dropwise on the sample to cover the sample, incubate for 30 min at room temperature away from light; (refer to the following table for the click reaction system, add each reagent in turn, mixing well while adding, and the amount of preparation can be increased or decreased proportionally, and it is recommended to prepare it beforehand)

Component	Volume
Reaction Buffer A	925 μL
Reaction Buffer B	10 μL
iF594 azide dye	15 μL
Reaction additive（Reagent C）	50 μL
Total volume	1000 μL

3.7. Remove the click reaction solution and immediately wash with PBS buffer 2-3 times for 5 min each;

3.8. Gently wipe off the PBS solution around the sample with filter paper;

3.9. **Nuclear staining**: Samples are stained in a staining vat, where slides are immersed in the dark into a staining vat containing DAPI solution (freshly prepared and diluted with PBS) and left at room temperature for 8 min (or nuclear staining is performed with Hoechst 33258);

3.10. **Sealing**: After the samples are stained, the tissue samples are washed three times with PBS for 5 min each time, then the excess liquid is gently removed and the samples are sealed with drops of anti-fluorescence quenching sealer (recommended G1401);

3.11. **Microscopy**: Immediately analyze the samples under a fluorescence microscope, slides are protected from light, DAPI stains both apoptotic and non-apoptotic cells blue, and only in the nuclei of apoptotic cells there is a red fluorescence localized by iF488 azide dye admixture.

Servicebio® Live or Dead Bacterial/Yeast Viability Kit（SYTO-9/PI）

Cat. No.：G1521

Product Information

Product Name	Cat. No.	Spec..
Live or Dead Bacterial Viability Kits（SYTO-9/PI）	G1521-20UL	20 μL

Product Description/Introduction

Live or Dead Bacterial Viability kit（SYTO-9/PI） is a convenient kit that uses SYTO-9 green nucleic acid dye and Propidium Iodide (PI) red fluorescent nucleic acid dye to detect bacterial viability. It can be analyzed by fluorescent analysis, such as Fluorescence Microplate Readers,flow cytometry and fluorescence microscope. Applicable to a variety of Gram-negative and Gram-positive bacteria, including Escherichia coli, Bacillus subtilis, micrococcus lysoides, etc. In addition, this kit can also be used for staining the vitality of yeast.

The kits contain two fluorescent dyes, SYTO-9 and Propidium Iodide (PI). SYTO-9 is a green nucleic acid fluorescent dye that can stain bacteria with intact and damaged membranes. PI is a red nucleic acid fluorescent dye that stains only dead bacteria with damaged membranes, but PI was added causes a decrease in the fluorescence of SYTO-9 staining. When two dyes were added to the bacterial suspension at the same time, appropriate adjustment of the ratio of SYTO-9 and PI could make the bacteria with intact membrane structure fluoresce green, while the bacteria with damaged membrane structure fluoresce red. The maximum excitation and emission wavelengths of the two dyes combined with nucleic acid were 485/530nm (SYTO-9) and 485/630nm (PI), respectively.

Storage and Shipping Conditions

Pack transportation with Wet ice ; Store at -20 ℃ and protected from light for 12 months.

Product Content

Component Number	Component	G1521
G1521-1	Propidium Iodide (PI)	20 μL
G1521-2	SYTO-9	20 μL
Specification		1

Number of Tests Possible

At the recommended reagent dilutions and volumes, kit contains sufficient material to perform:

40 individual tests by flow cytometry assay: 500 μL bacterial suspension was added to 1 μL dye mixture (SYTO 1 μL +PI 1 μL);

200 individual tests by Microplate Readers and fluorescence microscope assay: 100 μL bacteria solution was added to 1 μL diluted dye (SYTO 1 μL+ PI 1 μL+0.85% Nacl 8 μL).

Assay Protocol / Procedures

【Note】： Nucleic acids and other media components may bind to SYTO-9 and PI in an unpredictable manner, resulting in a large difference stainning from the expected results. Therefore, it is necessary to remove media residues, and phosphate buffers appear to decrease staining efficiency, 0.85% Nacl solution is recommended.During the experimental process, if it is found that the green fluorescence staining efficiency is too low, or the red fluorescence is too high, the fluorescence intensity can be adjusted to the expected effect by appropriately increasing the volume of the green dye or reducing the volume of the red dye.

Preparation of live/dead bacteria（eg.Escherichia coli, reference for bacterial viability ratio, optional）

(1) Escherichia coli was cultured to late log phase in LB nutrient broth.

(2) The bacteria was divided into two parts with EP tubes, and one part was centrifuged at 8000g for 10 min (the other part was placed at room temperature to prepare live bacteria).

(3) Remove the supernatant and resuspend the pellet in appropriate amount of 0.85% NaCl ,then add an appropriate amount of ethanol to make the final concentration of 70% ethanol mix thoroughly. That need incubate at room temperature for 1 h, mixing every 15 minutes prepare for killed bacteria.

(4) Concentrate both samples by centrifugation at 8000×g for 10 minutes.

(5) Remove the supernatant, add an appropriate of 0.85% NaCl to the two samples to resuspend the bacteria, and centrifuge again as in step 4, and resuspend.

(6) Determine the optical density at 670 nm (OD670) of both samples by spectrophotometer.

(7) The optical density of the two bacterial suspensions (live and killed) at OD670 was 0.03~0.1.

(8) As shown below to obtain the desired living cell/killed cell ratio by mix the two bacterial suspensions .

Live bacteria：killed bacteria	Volume of live bacteria（ml）	Volume of killed bacteria（ml）
0：100	0	1
20：80	0.2	0.8
50：50	0.5	0.5
80：20	0.8	0.2
100：0	1	0

Procedure for fluorescence microscopy

(1) Centrifuge the bacteria at 8000 g for 10 minutes, remove the supernatant, add an appropriate amount of 0.85% NaCl to resuspend the bacteria, repeat centrifugation again, and resuspend in an appropriate amount of 0.85% NaCl.

(2) Adjust the bacterial optical density, such as E. coli, with OD670 between 0.03 and 0.1.

(3) 1 volume of SYTO-9 and 1 volume of PI were mixed thoroughly in a microfuge tube.Then 8 volumes of 0.85% NaCl solution were added to obtain 100× dye solution.

(4) For every 100 µL bacterial suspension, add 1 µL of 100× dye solution.

(5) Mix thoroughly and incubate at room temperature for 15 minutes, away from light.

(6) Take 5µL of stained bacterial suspension drops on the slide, and then gently cover it with an 18 mm square cover slide.

(7) The live bacteria (green fluorescence) and dead bacteria (red fluorescence) can be observed and imaged using the FITC and Cy3 channels under a fluorescence microscope, respectively.

Procedure for Microplate Readers

(1) Centrifuge the bacteria at 8000 g for 10 minutes, remove the supernatant, add an appropriate amount of 0.85% NaCl to resuspend the bacteria, repeat centrifugation again, and resuspend in an appropriate amount of 0.85% NaCl.

(2) Adjust the bacterial optical density, such as E. coli, with OD670 between 0.03 and 0.1.

(3) 1 volume of SYTO-9 and 1 volume of PI were mixed thoroughly in a microfuge tube.Then 8 volumes of 0.85% NaCl solution were added to obtain 100× dye solution.

(4) Add 1 µL of 100× dye solution to every 100 µL of bacterial suspension in a flat-bottom 96-well plate.

(5) Mix thoroughly and incubate at room temperature for 15 minutes away from light.

(6) Fluorescence measurement and data analysis

a. Measure the fluorescence of each well using an excitation wavelength of ~485 nm and an emission wavelength of ~530 nm (emission 1, green);

b. Measure the fluorescence of each well using an excitation wavelength of ~485 nm and an emission wavelength of ~630 nm (emission 2, red);
c. Alculate the fluorescence ratio = emission 1 / emission 2;
d. Plot a linear graph with the proportion of live E. coli cells in the suspension as the x-axis and the emission 1/emission 2 ratio as the y-axis.

Procedure for flow cytometry

(1) Centrifuge the bacteria at 8000 g for 10 minutes, remove the supernatant, add an appropriate amount of 0.85% NaCl to resuspend the bacteria, repeat centrifugation again, and resuspend in an appropriate amount of 0.85% NaCl.
(2) Adjust the bacterial optical density, such as E. coli, with OD670 between 0.03 and 0.1.
(3) 1 volume of SYTO-9 and 1 volume of PI were mixed thoroughly in a microfuge tube.Then 8 volumes of 0.85% NaCl solution were added to obtain 100× dye solution.
(4) Add 5 μL of the dye mixture to every 500 μL of bacterial suspension.
(5) Mix thoroughly and incubate at room temperature for 15 minutes, away from light.
(6) For flow cytometry analysis: Use the FITC channel to detect the SYTO-9 fluorescence (green, live cells) and Use the PE/PC5.5 channel to detect the propidium iodide (PI) fluorescence (red, dead cells).

Caution

1. Since the SYTO-9 and propidium iodide (PI) components are limited, be sure to centrifuge at low speed before opening the cap to avoid loss.
2. After first-time use, you can aliquot the dyes into smaller portions for storage to avoid repeated freeze-thaw cycles and contamination.
3. SYTO-9 and PI are both storage solutions with a capacity of 1000×, which have been optimized to be suitable for most bacteria. However, in order to obtain more satisfactory results, please conduct certain concentration tests for different types of bacteria. The final concentration for the use of SYTO-9 and PI is generally 0.5-2×, with the highest recommended final concentration being 1×. For example, when yeast staining, SYTO-9 recommends a final concentration of 1 × and PI recommends a final concentration of 2×.
4. For E. coli, the recommended optical density (OD670) is 0.03-0.1. For Bacillus subtilis spores, the recommended OD670 is 0.01-0.1. For Micrococcus luteus, the recommended OD670 is 0.01-0.1. For yeast, the recommended OD670 is 0.1-0.3.
5. For your safety and health, please wear a lab coat and disposable gloves when operating.

Servicebio® Cell Cycle and Apoptosis Detection Kit

Cat. #: G1700-50T

Product Information

Product Name	Cat. No	Spec.
Cell Cycle and Apoptosis Detection Kit	G1700-50T	50 T

Product Description/Introduction

Cell cycle refers to the whole process of the cell from the completion of one division to the end of the next division, which is mainly divided into two stages: intermitotic phase and mitotic phase (M phase). The intermitotic phase is mainly composed of the early phase of DNA synthesis (G1 phase), DNA synthesis (S phase) and the late phase of DNA synthesis (G2 phase). The sequence of the whole cell cycle can be expressed as G1→S→G2→M. First of all, in G1 phase, cells mainly synthesize RNA and proteins to prepare materials and energy for cells to enter S phase. Then it enters S phase: the cells begin to synthesize DNA and some histones and other substances, and the cell DNA content begins to increase. Finally, the G2 phase: at this time, the DNA content of the cell has become twice that of the G1 phase, and has stopped DNA replication to enter the mitotic phase to do a lot of protein and other material synthesis; If the cell is in the G0 (Cells temporarily stop dividing, differentiating, and quiescence)/G1 phase, the DNA content of the cell is 1N; So the DNA content of the cell in G2 phase is 2N; The DNA content of S-phase cells between G1 and G2 is 1N to 2N. In apoptotic cells, the nucleus will be concentrated and DNA fragmentation will occur, leading to the loss of part of genomic DNA fragments, so the DNA content is less than 1N, and the so-called sub-G1 peak, namely the apoptotic cell peak, appears on the fluorogram of the flow cytometry. Therefore, the cell cycle and state can be judged according to the content of cell DNA.

Apoptosis can also be detected by observing changes in cell light scattering by flow cytometry. Apoptosis occurs when cells produce apoptotic bodies as a result of cytoplasmic and chromatin condensation and nuclear fragmentation. Chromatin shrinks and cell density increases in pre-apoptosis stage. In the late stage of apoptosis, cells produce apoptotic bodies, and the light scattering of cells changes.

The Cell Cycle and Apoptosis Analysis Kit use the classic Propidium staining method to detect and analyze Cell Cycle and Apoptosis. Propidium iodide is able to embed in double-stranded DNA and cause it to fluoresce. The cycle and state of a cell can be distinguished by the characteristic that the fluorescence intensity is proportional to the content of double-stranded DNA and the regular change of DNA content in different cell cycles. This kit can be used for cell cycle and apoptosis detection of tissue cells, adherent or suspended cells (if used for cell cycle and apoptosis detection of tissue, the tissue must be digested into a single cell state before detection).

Storage and Shipping Conditions

Ship with wet ice ; Store at -20℃ away from light, dyeing buffer store at 4℃, valid for 12 months.

Product Components

Component Number	Component	G1700-50T
G1700-1	PI Staining Solution (50×)	500 μL

G1700-2	RNase A (50×)	500 μL
G1700-3	Staining Buffer	25 mL
Manual		1 pc

Note

1. Cell culture medium containing serum.
2. Trypsin digestion solution (G4001 is recommended).
3. PBS buffer (G4202 is recommended).
4. 75% ethanol.

Product Protocol/Procedures

1. Preparation of Cell Samples (the number of cells is controlled at 1×10^5~1×10^6)

a) **For adherent cells:** remove the culture medium, add trypsin digestion solution to digest the cells, and observe that the cells become round and loose under the microscope. Add the appropriate amount of cell medium contains serum to terminate the digestion, gently blow the cells apart and make a suspension of the cells. transfer the suspension to a centrifuge tube, centrifuge at 1000 x g for 3-5 min, discard the supernatant and retain the cell precipitation. Then wash the cell precipitation 1-2 times with pre-cooled PBS buffer, and discard the supernatant by centrifugation in the same way to retain the cell precipitation.

b) **For the suspended cells:** transfer the cells to a centrifuge tube, centrifuge at 1000 x g for 3-5 min, discard the supernatant, and retain the cell precipitation; Wash the cell precipitation 1-2 times with pre-cooled PBS buffer, and discard the supernatant by centrifugation in the same way to retain the cell precipitation.

c) **For tissue cells:** Cut the tissue into small pieces as much as possible, digest the pieces with digestive enzymes such as trypsin and collagenase according to the source of the tissue, and filter the tissue through a 100-300 mesh sieve to obtain a single cell suspension; transfer the filtered cell suspension to a centrifuge tube, centrifuge at 1000 x g for 3-5 min, discard the supernatant and retain the cell precipitation; then wash the cell precipitate 1-2 times with pre-cooled PBS buffer, discard the supernatant by centrifuge in the same way to retain the cell precipitation.

2. Fixation of Cell Samples

a) Add 1 mL of 75% pre-cooled ethanol on ice to the collected cell precipitation sample, and gently blow the cells to make them thoroughly mixed.

b) Cells are fixed at 4°C for 30 min or longer (usually 2 h or more is better for staining, 12-24 h may be better to improve staining effect).

c) After fixed for a certain time, the cells are centrifuged at 1000 x g for 3-5 min to remove the ethanol fixing solution and retain the cell precipitation.

d) Tap the bottom of the centrifuge tube to disperse the cells, resuspend and wash the cells with PBS buffer, centrifuge at 1000 x g for 3-5 min, discard the supernatant and collect the cell precipitation.

3. Preparation and Staining of Working Solution

a) According to the following table, the dyeing solution can be prepared out of light, and the amount can be increased or decreased in equal proportion according to the use requirements

(the prepared dyeing solution can be stored at 4℃ in a short time, please use it within the same day).

	1 Sample	5 Samples	10 Samples
Staining Buffer	480 μL	2.4 mL	4.8 mL
PI Stain (50×)	10 μL	50 μL	100 μL
RNaseA (50×)	10 μL	50 μL	100 μL
Total Volume	500 μL	2.5 mL	5 mL

b) Tap the bottom of the centrifuge tube to disperse the cells precipitation in Step 2.4, then add 500 μL of the prepared staining working solution, and gently blow to disperse the cells and mix with the staining working solution.

c) Incubate at 37℃ for 30 min in the dark, use flow cytometry for detection.

4. Flow Detection and Analysis

A flow cytometer is used to detect red fluorescence at an excitation wavelength of 488 nm, together with light scattering. Cell DNA content analysis and light scattering analysis are performed using appropriate analysis software.

Note

1. Fluorescent dyes are subject to fluorescence quenching and should be protected from light during use and storage.
2. It is recommended to synchronise the cells before the experiment to avoid large reproducibility differences caused by different cell cycles.
3. The planting density of experimental cells should not be too high or too low to prevent contact inhibition or density dependence.
4. Protect from direct contact with humans or inhalation when handling PI staining solution.
5. For your safety and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® Click-iT EdU-488 Cell Proliferation Detection Kit

Cat. #：G1601

Product information

product name	Identification of product	model
Click-iT EdU-488 Cell Proliferation Detection Kit	G1601	100T

Descirption/Introduction

Analyzing cell proliferation ability is a common and important evaluation method in life sciences. It can judge the influence of certain genes, drugs, etc. on cells cultured in vitro, or analyze the growth and renewal ability of tissue cells under different conditions or stimulation. At present, there are many methods to detect cell proliferation. Most of them use some metabolic enzymes produced by cells to indirectly assess cell proliferation activity (such as CCK-8 method, MTT method, etc.), but some drugs or the state of the cell itself will have a certain impact on the results of the assessment. Direct detection of DNA synthesis in cells to determine cell proliferation is recognized as the most accurate and effective detection method. However, both the original radiolabeled nucleoside incorporation method and the subsequent improvement of the BrdU method based on antibody detection have their own limitations.

EdU (5-Ethynyl-2'-deoxyuridine, 5-ethynyl-2'-deoxyuridine) is a thymidine analogue containing an acetylene group, when injected into animals or incubating cells cultured in vitro, these small molecules can quickly diffuse to various organs and tissues, and infiltrate into the cells, and can replace thymidine (T) into newly synthesized DNA during cell proliferation. The acetylene group in the EdU molecule can react with the fluorescently iF488 labeled azide compound probe to form a stable triazole ring under the catalysis of copper ions, so the newly synthesized DNA can be labeled with the corresponding fluorescent probe. Compared with the radiolabeled nucleoside incorporation method, the EdU detection method has no limiting factors such as radioactive contamination; compared with the BrdU detection method, the EdU detection method does not require DNA denaturation or antigen-antibody reaction, which greatly reduces the complexity of the experiment and also make the experiment more time-saving, more sensitive, more stable and more accurate.

This kit can be used to detect cell proliferation in cultured cells or animal tissues. The fluorescent probe in this kit is green fluorescence, the maximum excitation wavelength is 491 nm, and the maximum emission wavelength is 516 nm. After the proliferating cells are labeled, the cell nucleus will show bright green fluorescence, and the cell nucleus will be jointly labeled with the matching conventional nuclear dye (This kit provides Hoechst 33342 cell nuclear dye), you can use fluorescence microscope, laser confocal microscope and other instruments to directly observe cell proliferation; you can also use flow cytometry to detect the fluorescence intensity of cultured cells in vitro, and then determine the cell cycle based on the fluorescence intensity DNA replication activity in mid-S phase.

Storage and Handling Conditions

Reagent B(iF488 dye) should be store at -20°C away from light;
EdU catalytic reagent (Reagent A) and reaction buffer can be stored at 4°C;
Valid for 12 months.

Component

Component Number	Component	G1601-100T

G1601-1	EdU storage solution（10 mM）	100 μL
G1601-2	Reagent A	120 μL
G1601-3	Reagent B (iF488 dye)	50 μL
G1601-4	Reagent C	2 × 100 mg (powder)
G1601-5	reaction buffer	20 mL
G1601-6	Hoechst 33342 Staining Solution	30 μL
Manual		1 pc

Note: The above reaction times are for the corresponding 96-well plate assay.

Experiment preparation

1. Cell culture medium containing serum;
2. Permeabilization solution: 0.2-0.5% Triton X-100 in PBS(recommend our product, Cat.#:G1204);
3. Fixiative solution: 4% paraformaldehyde in PBS, pH 7.4 (recommend our product,Cat.#:G1101);
4. PBS buffer (recommend our product,Cat.#:G4202);
5. Ultrapure water;
6. Animal modeling and tissue sectioning related reagents (animal tissue cell proliferation assay).

Assay Protocol / Procedures

1. Pretreatment of cultured cells in vitro:

1.1. Plant the cells evenly in the cell culture plate at a certain density (the planting density is determined by factors such as cell size, growth speed, etc.). After the cells adhere to the wall or return to a normal state, perform corresponding drug stimulation and other treatments. (For suspension cells, please follow the normal operation method of suspension cells. The whole experiment needs to add centrifugation and other steps).

1.2. The catalytic additive (Reagent C) was centrifuged at low speed, 100 mg was taken and dissolved by adding 1 mL of ultrapure water and dispensed and stored at -20°C, the remaining powder was kept as reserve.

2. In vitro cellular EdU labeling, fixation and permeabilization:

2.1. Prepare **2×EdU incubation working solution:** add 2 μL **EdU storage solution (10 mM)** to every 1 mL of complete cell culture medium, which is 20 μM **2×EdU incubation working solution**, and put it in the incubator to preheat (the recommend EdU working concentration is 10 μM for preliminary experiments);

2.2. In the half-changing mode, aspirate half of the original cell culture medium in the culture plate, and add an equal volume of preheated **2×EdU incubation working solution**, and incubate for a certain period of time (the duration of the incubation generally depends on the growth cycle of the corresponding cells, which usually accounts for about 10% of the cell cycle. For mostly adherent and fast-growing cells, incubation for about 2 h is recommended. For specific cases, it needs to be adjusted with the cell characteristics, the actual situation after treatment, etc. If a longer incubation time is required, the EdU working concentration can be appropriately reduced; for a shorter time, the EdU concentration can be appropriately increased);

2.3. After EdU incubation, wash with PBS buffer for 1-2 times, add fixing fluid to cover the cells, and fix at room temperature for 15 minutes (if flow cytometry is required, the adherent cells should be digested and resuspended before this step fix, follow the suspension cell processing method); Wash 2-3 times with PBS buffer, 3-5 min each time;

2.4. Remove the PBS buffer, add permeabilization solution to cover the cells, and incubate at room

temperature for 15 minutes;

2.5. Remove the permeabilization solution, wash 1-2 times with PBS buffer, 3-5 min each time. Then go to step 4.

3. Animals EdU injection modeling as well as tissue section processing:

3.1. According to experimental requirements, one or more EdU injections are used to model animals by intraperitoneal injection, intramuscular injection, subcutaneous injection, tail vein injection, etc. Generally, the ratio of EdU dosage to animal body weight is 5 mg/kg, the actual injection dose depends on the research content and animal conditions. The EdU storage solution provided in this kit is mainly used for in vitro cell EdU labeling. If you need to model an animal with EdU, you can order the EdU reagent separately (Cat. No.: G5059);

3.2. Epithelial cells such as the small intestine proliferate quickly, while brain cells proliferate slowly. The faster-growing tissues usually take less than 12 hours for labeling, while those slower-growing tissues may take several days for labeling.The optimal labeling time was determined according to the specific experiment. Due to the rapid proliferation of intestinal epithelial tissue, such tissue was recommended as a reference for labeling.

3.3. After the animal is killed according to the specified standards, the tissues needed are taken out and frozen sections or paraffin sections are made according to the conventional procedures:

a. **For frozen sections:** return the sections to room temperature, add an appropriate amount of Fixing fluid, and fix at room temperature for 15 minutes. Remove the Fixing fluid and wash 3 times with PBS buffer for 3-5 min each; remove the PBS buffer and cover the tissue with an appropriate amount of permeabilization solution and incubate at room temperature for 10-15 min; remove the permeabilization solution and wash with PBS buffer 1- 2 times, 3-5 min each time. Then go to step 4.

b. **For paraffin sections:** Deparaffinize and rehydrate the sections, and wash with PBS for 5 min. Remove the PBS buffer, add permeabilization solution to cover the cells or tissues, and incubate at room temperature for 15 min; Then wash with PBS buffer for 1-2 times, each time for 3-5 min. Then go to step 4.

4. EdU click reaction:

4.1. During cell or tissue fixation and perforation, preparation of reaction solution: mix the reagents according to the following ratio, the volume of preparation can be increased or decreased in proportion to the number of samples.

Component	Volume (for cell)	Volume (for Histological section)
reaction buffer	935 μL	928 μL
Reagent A	10 μL	10 μL
Reagent B (iF488 dye)	5 μL	12 μL
Reagent C	50 μL	50 μL
total capacity	**1000 μL**	**1000 μL**

4.2. Remove the PBS buffer from the cells or sections, add the reaction solution, shake gently to ensure that the reaction solution covers all the cells or tissues, and incubate for 30 min at room temperature in the dark;

4.3. Remove the reaction solution, wash 2-3 times with PBS buffer, 3-5 min each time (If there is no other special requirement, the fluorescence intensity can be detected by flow cytometry or the fluorescence effect can be detected by other instruments).

5. Nuclear stain(optional):

5.1. Dilute Hoechst 33342 staining solution with PBS buffer at a ratio of 1:500-1000, add to the

sample to cover the cells, and incubate for 5 min;

5.2. Remove Hoechst 33342 staining solution, wash 2-3 times with PBS buffer, 3-5 min each time.

6. Imaging and detection analysis:

Use fluorescence microscope or confocal microscope to detect processed cells or tissue section samples, and analyze the proportion of proliferating cells. Alternatively, cells cultured in vitro can be collected and the fluorescence intensity can be measured by flow cytometry (it is recommended to use cell samples not labeled with EdU as a negative control for the flow cytometry assay and to choose the appropriate voltage), and based on the fluorescence intensity, the DNA replication activity of the S-phase in the cell cycle can be determined. The fluorescent dye iF488 (Reagent B) in this kit corresponds to the spectral characterization of Ex/Em: 491 nm/516 nm (green); Hoechst 33342 staining solution corresponds to the spectral characterization of Ex/Em: 346 nm/460 nm (blue).

Note:

1. For cultured cells, the specific EdU concentration and incubation time can be adjusted appropriately depending on the sample and research purpose.
2. Some tissue cells proliferate slowly. In order to avoid poor modeling effect, it is recommended to select tissue samples with fast proliferation as reference samples (such as intestinal tissue).
3. If the background color is too dark, it may be caused by insufficient washing, a long time fixed, and residual fixative, etc.in the experiment.
4. Reagent C (EdU catalytic addition reagent) is easy to oxidize. Try to avoid prolonged exposure to the air. After being prepared as an aqueous solution, it is recommended to store in aliquot; Tested, such as EdU catalytic additive reagent color changes slightly, click reaction catalytic system is still able to proceed normally. if reagent C appears brown, it indicates that the component has expired, so please discard it.
5. For your health and safety, please wear lab coats and gloves during operation.

This product is for research only, not for clinical diagnosis!

version number: V1.0-202103

Servicebio® Trypan Blue Stain

Cat No.: G1019-10ML

Product Information

Product Name	Cat.No.	Spec.
Trypan Blue Stain	G1019-10ML	10 mL

Description

Trypan Blue is a reactive dye for cells, which is often used to detect the integrity of cell membranes. Because the envelope of normal living cells is complete, it can repel trypan blue, making it unable to enter the cells, while the permeability of dead cells or cells with incomplete envelope increases, and can be dyed blue by trypan blue. Therefore, the cell survival rate can be determined simply and quickly by trypan blue staining. This product is trypan blue dye solution with a concentration of 0.4%, which is used for living cell dyeing and counting.

Storage and Handling Conditions

Wet ice transportation; Store at 2-8℃ for 12 months.

Component

Component	G1019
Trypan Blue Stain	10 mL
Product Manual	

Assay Protocol

1. The adherent cells were digested by trypsin to prepare single cell suspension and diluted appropriately.
2. Staining: Mix the cell suspension with trypan blue dye at a volume ratio of 9:1, and dye for 3-5 min.
3. Observe under the microscope, or take 10 μ L was added to the cell counting plate, and the cell counter was used for automatic counting. The dead cells were dyed obviously blue, and the living cells were refused to be dyed colorless and transparent.
4. Counting: count living cells and dead cells respectively within three minutes.
5. Statistics of cell vitality: living cell rate (%)=number of living cells/(number of living cells+number of dead cells) × 100%。

Servicebio® Click-iT EdU-555 Cell Proliferation Assay Kit

Cat.#：G1602

Product information

product name	Identification of product	model
Click-iT EdU-555 Cell Proliferation Assay Kit	G1602	100T

Description/Introduction

Analyzing cell proliferation ability is a common and important evaluation method in life sciences. It can judge the influence of certain genes, drugs, etc. on cells cultured in vitro, or analyze the growth and renewal ability of tissue cells under different conditions or stimulation. At present, there are many methods to detect cell proliferation. Most of them use some metabolic enzymes produced by cells to indirectly assess cell proliferation activity (such as CCK-8 method, MTT method, etc.), but some drugs or the state of the cell itself will have a certain impact on the results of the assessment. Direct detection of DNA synthesis in cells to determine cell proliferation is recognized as the most accurate and effective detection method. However, both the original radiolabeled nucleoside incorporation method and the subsequent improvement of the BrdU method based on antibody detection have their own limitations.

EdU (5-Ethynyl-2'-deoxyuridine, 5-ethynyl-2'-deoxyuridine) is a thymidine analogue containing an acetylene group, when injected into animals or incubating cells cultured in vitro, these small molecules can quickly diffuse to various organs and tissues, and infiltrate into the cells, and can replace thymidine (T) into newly synthesized DNA during cell proliferation. The acetylene group in the EdU molecule can react with the fluorescently iF488 labeled azide compound probe to form a stable triazole ring under the catalysis of copper ions, so the newly synthesized DNA can be labeled with the corresponding fluorescent probe. Compared with the radiolabeled nucleoside incorporation method, the EdU detection method has no limiting factors such as radioactive contamination; compared with the BrdU detection method, the EdU detection method does not require DNA denaturation or antigen-antibody reaction, which greatly reduces the complexity of the experiment and also make the experiment more time-saving, more sensitive, more stable and more accurate.

This kit can be used to detect cell proliferation in cultured cells or animal tissues. The fluorescent probe in this kit is red fluorescence, the maximum excitation wavelength is 557 nm, and the maximum emission wavelength is 570 nm. After the proliferating cells are labeled, the cell nucleus will show bright red fluorescence, and the cell nucleus will be jointly labeled with the matching conventional nuclear dye (This kit provides Hoechst 33342 cell nuclear dye), you can use fluorescence microscope, laser confocal microscope and other instruments to directly observe cell proliferation; you can also use flow cytometry to detect the fluorescence intensity of cultured cells in vitro, and then determine the cell cycle based on the fluorescence intensity DNA replication activity in mid-S phase.

Storage and Handling Conditions

Transport by wet ice; EdU catalytic reagent (Reagent A) and reaction buffer can be stored at 4℃， Valid for 12 months.

Component

Component Number	Component	G1602-100T

G1602-1	EdU storage solution (10 mM)	100 μL
G1602-2	Catalytic reagent (Reagent A)	120 μL
G1602-3	Fluorescent dye iF555 (reagent B)	50 μL
G1602-4	Catalytic additive (Reagent C)	2×100 mg (powder)
G1602-5	Reaction buffer	20 mL
G1602-6	Hoechst 33342 staining solution	30 μL
Manual		One copy

Materials required but not provided

1. Cell culture medium containing serum;
2. **Permeabilization reagent:**0.2%- 0.5% Triton® X-100 in PBS (G1204);
3. **Fixative:** 4% paraformaldehyde (G1101) or other similar reagents;
4. **Phosphate-buffered saline (PBS)**;
5. **Ultrapure water**;
6. Animal modeling and tissue section related reagents (animal tissue cell proliferation detection)

Assay Protocol / Procedures

1. Pretreatment of cultured cells in vitro:

1.1. Plant the cells evenly in the cell culture plate at a certain density (the planting density is determined by factors such as cell size, growth speed, etc.). After the cells adhere to the wall or return to a normal state, perform corresponding drug stimulation and other treatments. (For suspension cells, please follow the normal operation method of suspension cells. The whole experiment needs to add centrifugation and other steps).

1.2. The catalytic additive (Reagent C) was centrifuged at low speed, 100 mg was taken and dissolved by adding 1 mL of ultrapure water and dispensed and stored at -20°C, the remaining powder was kept as reserve.

2. In vitro cellular EdU labeling, fixation and permeabilization:

2.1. Prepare **2×EdU incubation working solution:** add 2 μL **EdU storage solution (10 mM)** to every 1 mL of complete cell culture medium, which is 20 μM **2×EdU incubation working solution**, and put it in the incubator to preheat (the recommend EdU working concentration is 10 μM for preliminary experiments);

2.2. In the half-changing mode, aspirate half of the original cell culture medium in the culture plate, and add an equal volume of preheated **2×EdU incubation working solution**, and incubate for a certain period of time (the duration of the incubation generally depends on the growth cycle of the corresponding cells, which usually accounts for about 10% of the cell cycle. For mostly adherent and fast-growing cells, incubation for about 2 h is recommended. For specific cases, it needs to be adjusted with the cell characteristics, the actual situation after treatment, etc. If a longer incubation time is required, the EdU working concentration can be appropriately reduced; for a shorter time, the EdU concentration can be appropriately increased);

2.3. After EdU incubation, wash with PBS buffer for 1-2 times, add fixing fluid to cover the cells, and fix at room temperature for 15 minutes (if flow cytometry is required, the adherent cells should be digested and resuspended before this step fix, follow the suspension cell processing method); Wash 2-3 times with PBS buffer, 3-5 min each time;

2.4. Remove the PBS buffer, add permeabilization solution to cover the cells, and incubate at room temperature for 15 minutes;

2.5. Remove the permeabilization solution, wash 1-2 times with PBS buffer, 3-5 min each time. Then

go to step 4.

3. Animals EdU injection modeling as well as tissue section processing:

3.1. According to experimental requirements, one or more EdU injections are used to model animals by intraperitoneal injection, intramuscular injection, subcutaneous injection, tail vein injection, etc. Generally, the ratio of EdU dosage to animal body weight is 5 mg/kg, the actual injection dose depends on the research content and animal conditions. The EdU storage solution provided in this kit is mainly used for in vitro cell EdU labeling. If you need to model an animal with EdU, you can order the EdU reagent separately (Cat. No.: G5059);

3.2. Epithelial cells such as the small intestine proliferate quickly, while brain cells proliferate slowly. The faster-growing tissues usually take less than 12 hours for labeling, while those slower-growing tissues may take several days for labeling.The optimal labeling time was determined according to the specific experiment. Due to the rapid proliferation of intestinal epithelial tissue, such tissue was recommended as a reference for labeling.

3.3. After the animal is killed according to the specified standards, the tissues needed are taken out and frozen sections or paraffin sections are made according to the conventional procedures:

a. **For frozen sections:** return the sections to room temperature, add an appropriate amount of Fixing fluid, and fix at room temperature for 15 minutes. Remove the Fixing fluid and wash 3 times with PBS buffer for 3-5 min each; remove the PBS buffer and cover the tissue with an appropriate amount of permeabilization solution and incubate at room temperature for 10-15 min; remove the permeabilization solution and wash with PBS buffer 1- 2 times, 3-5 min each time. Then go to step 4.

b. **For paraffin sections:** Deparaffinize and rehydrate the sections, and wash with PBS for 5 min. Remove the PBS buffer, add permeabilization solution to cover the cells or tissues, and incubate at room temperature for 15 min; Then wash with PBS buffer for 1-2 times, each time for 3-5 min. Then go to step 4.

4. EdU click reaction:

4.1. During cell or tissue fixation and perforation, preparation of reaction solution: mix the reagents according to the following ratio, the volume of preparation can be increased or decreased in proportion to the number of samples.

Component	Volume (for cell)	Volume (for Histological section)
reaction buffer	935 μL	928 μL
Reagent A	10 μL	10 μL
Reagent B (iF555 dye)	5 μL	12 μL
Reagent C	50 μL	50 μL
total capacity	**1000 μL**	**1000 μL**

4.2. Remove the PBS buffer from the cells or sections, add the reaction solution, shake gently to ensure that the reaction solution covers all the cells or tissues, and incubate for 30 min at room temperature in the dark;

4.3. Remove the reaction solution, wash 2-3 times with PBS buffer, 3-5 min each time (If there is no other special requirement, the fluorescence intensity can be detected by flow cytometry or the fluorescence effect can be detected by other instruments).

5. Nuclear stain(optional):

5.1. Dilute Hoechst 33342 staining solution with PBS buffer at a ratio of 1:500-1000, add to the sample to cover the cells, and incubate for 5 min;

5.2. Remove Hoechst 33342 staining solution, wash 2-3 times with PBS buffer, 3-5 min each time.

6. Imaging and detection analysis:

Use fluorescence microscope or confocal microscope to detect processed cells or tissue section samples, and analyze the proportion of proliferating cells. Alternatively, cells cultured in vitro can be collected and the fluorescence intensity can be measured by flow cytometry (it is recommended to use cell samples not labeled with EdU as a negative control for the flow cytometry assay and to choose the appropriate voltage), and based on the fluorescence intensity, the DNA replication activity of the S-phase in the cell cycle can be determined. The fluorescent dye if555 (Reagent B) in this kit corresponds to the spectral characterization of Ex/Em: 557 nm/570 nm (red); Hoechst 33342 staining solution corresponds to the spectral characterization of Ex/Em: 346 nm/460 nm (blue).

Note:

1. For cultured cells, the specific EdU concentration and incubation time can be adjusted appropriately depending on the sample and research purpose.
2. Some tissue cells proliferate slowly. In order to avoid poor modeling effect, it is recommended to select tissue samples with fast proliferation as reference samples (such as intestinal tissue).
3. If the background color is too dark, it may be caused by insufficient washing, a long time fixed, and residual fixative, etc.in the experiment.
4. Reagent C (EdU catalytic addition reagent) is easy to oxidize. Try to avoid prolonged exposure to the air. After being prepared as an aqueous solution, it is recommended to store in aliquot; Tested, such as EdU catalytic additive reagent color changes slightly, click reaction catalytic system is still able to proceed normally. if reagent C appears brown, it indicates that the component has expired, so please discard it.
5. For your health and safety, please wear lab coats and gloves during operation.

Ver. No.: V1.0-202101

Servicebio® Click-iT EdU-594 Cell Proliferation Assay Kit

Cat. #：G1603

Product Information

Product Name	Cat. No	Spec.
Click-iT EdU-594 Cell Proliferation Assay Kit	G1603	100 T

Product Description/Introduction

Analyzing cell proliferation ability is a common and important evaluation method in life sciences. It can judge the influence of certain genes, drugs, etc. on cells cultured in vitro, or analyze the growth and renewal ability of tissue cells under different conditions or stimulation. At present, there are many methods to detect cell proliferation. Most of them use some metabolic enzymes produced by cells to indirectly assess cell proliferation activity (such as CCK-8 method, MTT method, etc.), but some drugs or the state of the cell itself will have a certain impact on the results of the assessment. Direct detection of DNA synthesis in cells to determine cell proliferation is recognized as the most accurate and effective detection method. However, both the original radiolabeled nucleoside incorporation method and the subsequent improvement of the BrdU method based on antibody detection have their own limitations.

EdU (5-Ethynyl-2'-deoxyuridine, 5-ethynyl-2'-deoxyuridine) is a thymidine analogue containing an acetylene group, when injected into animals or incubating cells cultured in vitro, these small molecules can quickly diffuse to various organs and tissues, and infiltrate into the cells, and can replace thymidine (T) into newly synthesized DNA during cell proliferation. The acetylene group in the EdU molecule can react with the fluorescently iF488 labeled azide compound probe to form a stable triazole ring under the catalysis of copper ions, so the newly synthesized DNA can be labeled with the corresponding fluorescent probe. Compared with the radiolabeled nucleoside incorporation method, the EdU detection method has no limiting factors such as radioactive contamination; compared with the BrdU detection method, the EdU detection method does not require DNA denaturation or antigen-antibody reaction, which greatly reduces the complexity of the experiment and also make the experiment more time-saving, more sensitive, more stable and more accurate.

This kit can be used to detect cell proliferation in cultured cells or animal tissues. The fluorescent probe in this kit is red fluorescence, the maximum excitation wavelength is 593 nm, and the maximum emission wavelength is 614 nm. After the proliferating cells are labeled, the cell nucleus will show bright red fluorescence, and the cell nucleus will be jointly labeled with the matching conventional nuclear dye (This kit provides Hoechst 33342 cell nuclear dye), you can use fluorescence microscope, laser confocal microscope and other instruments to directly observe cell proliferation; you can also use flow cytometry to detect the fluorescence intensity of cultured cells in vitro, and then determine the cell cycle based on the fluorescence intensity DNA replication activity in mid-S phase.

Storage and Shipping Conditions

Ship with wet ice; store at -20°C in the dark. Catalyst (Reagent A) and Reaction Buffer can be stored at 4°C; Valid for 12 months.

Product Contents

Component Number	Component	G1603-100T

G1603-1	EdU Stock Solution (10 mM)	100 μL
G1603-2	Catalyst (Reagent A)	120 μL
G1603-3	Fluorescent Stain iF594 (Reagent B)	50 μL
G1603-4	Catalytic Additive (Reagent C)	2×100 mg (powder)
G1603-5	Reaction Buffer	20 mL
G1603-6	Hoechst 33342	30 μL
Manual		One copy

Note: The above reaction times are corresponding to 96-well plate detection.

Before starting

1. Serum-containing cell culture medium;
2. Permeabilization buffer: buffer containing 0.2-0.5% Triton X-100 (recommended G1204);
3. Fixative: 4% paraformaldehyde (recommended G1101) or other similar reagents;
4. PBS buffer (recommended G4202);
5. Ultrapure water;
6. Animal modeling and tissue section related reagents (animal tissue cell proliferation detection).

Assay Protocol / Procedures

1. Pretreatment of cultured cells in vitro:

1.1. Plant the cells evenly in the cell culture plate at a certain density (the planting density is determined by factors such as cell size, growth speed, etc.). After the cells adhere to the wall or return to a normal state, perform corresponding drug stimulation and other treatments. (For suspension cells, please follow the normal operation method of suspension cells. The whole experiment needs to add centrifugation and other steps).

1.2. The catalytic additive (Reagent C) was centrifuged at low speed, 100 mg was taken and dissolved by adding 1 mL of ultrapure water and dispensed and stored at -20°C, the remaining powder was kept as reserve.

2. In vitro cellular EdU labeling, fixation and permeabilization:

2.1. Prepare **2×EdU incubation working solution:** add 2 μL **EdU storage solution (10 mM)** to every 1 mL of complete cell culture medium, which is 20 μM **2×EdU incubation working solution**, and put it in the incubator to preheat (the recommend EdU working concentration is 10 μM for preliminary experiments);

2.2. In the half-changing mode, aspirate half of the original cell culture medium in the culture plate, and add an equal volume of preheated **2×EdU incubation working solution**, and incubate for a certain period of time (the duration of the incubation generally depends on the growth cycle of the corresponding cells, which usually accounts for about 10% of the cell cycle. For mostly adherent and fast-growing cells, incubation for about 2 h is recommended. For specific cases, it needs to be adjusted with the cell characteristics, the actual situation after treatment, etc. If a longer incubation time is required, the EdU working concentration can be appropriately reduced; for a shorter time, the EdU concentration can be appropriately increased);

2.3. After EdU incubation, wash with PBS buffer for 1-2 times, add fixing fluid to cover the cells, and

fix at room temperature for 15 minutes (if flow cytometry is required, the adherent cells should be digested and resuspended before this step fix, follow the suspension cell processing method); Wash 2-3 times with PBS buffer, 3-5 min each time;

2.4. Remove the PBS buffer, add permeabilization solution to cover the cells, and incubate at room temperature for 15 minutes;

2.5. Remove the permeabilization solution, wash 1-2 times with PBS buffer, 3-5 min each time. Then go to step 4.

3. Animals EdU injection modeling as well as tissue section processing:

3.1. According to experimental requirements, one or more EdU injections are used to model animals by intraperitoneal injection, intramuscular injection, subcutaneous injection, tail vein injection, etc. Generally, the ratio of EdU dosage to animal body weight is 5 mg/kg, the actual injection dose depends on the research content and animal conditions. The EdU storage solution provided in this kit is mainly used for in vitro cell EdU labeling. If you need to model an animal with EdU, you can order the EdU reagent separately (Cat. No.: G5059);

3.2. Epithelial cells such as the small intestine proliferate quickly, while brain cells proliferate slowly. The faster-growing tissues usually take less than 12 hours for labeling, while those slower-growing tissues may take several days for labeling.The optimal labeling time was determined according to the specific experiment. Due to the rapid proliferation of intestinal epithelial tissue, such tissue was recommended as a reference for labeling.

3.3. After the animal is killed according to the specified standards, the tissues needed are taken out and frozen sections or paraffin sections are made according to the conventional procedures:

a. **For frozen sections:** return the sections to room temperature, add an appropriate amount of Fixing fluid, and fix at room temperature for 15 minutes. Remove the Fixing fluid and wash 3 times with PBS buffer for 3-5 min each; remove the PBS buffer and cover the tissue with an appropriate amount of permeabilization solution and incubate at room temperature for 10-15 min; remove the permeabilization solution and wash with PBS buffer 1- 2 times, 3-5 min each time. Then go to step 4.

b. **For paraffin sections:** Deparaffinize and rehydrate the sections, and wash with PBS for 5 min. Remove the PBS buffer, add permeabilization solution to cover the cells or tissues, and incubate at room temperature for 15 min; Then wash with PBS buffer for 1-2 times, each time for 3-5 min. Then go to step 4.

4. EdU click reaction:

4.1. During cell or tissue fixation and perforation, preparation of reaction solution: mix the reagents according to the following ratio, the volume of preparation can be increased or decreased in proportion to the number of samples.

Component	Volume (for cell)	Volume (for Histological section)
reaction buffer	935 μL	928 μL
Reagent A	10 μL	10 μL
Reagent B (iF594 dye)	5 μL	12 μL
Reagent C	50 μL	50 μL
total capacity	**1000 μL**	**1000 μL**

4.2. Remove the PBS buffer from the cells or sections, add the reaction solution, shake gently to ensure that the reaction solution covers all the cells or tissues, and incubate for 30 min at room temperature in the dark;

4.3. Remove the reaction solution, wash 2-3 times with PBS buffer, 3-5 min each time (If there is no

other special requirement, the fluorescence intensity can be detected by flow cytometry or the fluorescence effect can be detected by other instruments).

5. Nuclear stain(optional):

5.1. Dilute Hoechst 33342 staining solution with PBS buffer at a ratio of 1:500-1000, add to the sample to cover the cells, and incubate for 5 min;

5.2. Remove Hoechst 33342 staining solution, wash 2-3 times with PBS buffer, 3-5 min each time.

6. Imaging and detection analysis:

Use fluorescence microscope or confocal microscope to detect processed cells or tissue section samples, and analyze the proportion of proliferating cells. Alternatively, cells cultured in vitro can be collected and the fluorescence intensity can be measured by flow cytometry (it is recommended to use cell samples not labeled with EdU as a negative control for the flow cytometry assay and to choose the appropriate voltage), and based on the fluorescence intensity, the DNA replication activity of the S-phase in the cell cycle can be determined. The fluorescent dye iF594 (Reagent B) in this kit corresponds to the spectral characterization of Ex/Em: 593 nm/614 nm (red); Hoechst 33342 staining solution corresponds to the spectral characterization of Ex/Em: 346 nm/460 nm (blue).

Note:

1. For cultured cells, the specific EdU concentration and incubation time can be adjusted appropriately depending on the sample and research purpose.
2. Some tissue cells proliferate slowly. In order to avoid poor modeling effect, it is recommended to select tissue samples with fast proliferation as reference samples (such as intestinal tissue).
3. If the background color is too dark, it may be caused by insufficient washing, a long time fixed, and residual fixative, etc.in the experiment.
4. Reagent C (EdU catalytic addition reagent) is easy to oxidize. Try to avoid prolonged exposure to the air. After being prepared as an aqueous solution, it is recommended to store in aliquot; Tested, such as EdU catalytic additive reagent color changes slightly, click reaction catalytic system is still able to proceed normally. if reagent C appears brown, it indicates that the component has expired, so please discard it.
5. For your health and safety, please wear lab coats and gloves during operation.

Servicebio® Click-iT EdU-647 Cell Proliferation Assay Kit

Cat. #：G1604

Product Information

Product Name	Cat. No	Spec.
Click-iT EdU-647 Cell Proliferation Assay Kit	G1604	100 T

Product Description/Introduction

Analyzing cell proliferation ability is a common and important evaluation method in life sciences. It can judge the influence of certain genes, drugs, etc. on cells cultured in vitro, or analyze the growth and renewal ability of tissue cells under different conditions or stimulation. At present, there are many methods to detect cell proliferation. Most of them use some metabolic enzymes produced by cells to indirectly assess cell proliferation activity (such as CCK-8 method, MTT method, etc.), but some drugs or the state of the cell itself will have a certain impact on the results of the assessment. Direct detection of DNA synthesis in cells to determine cell proliferation is recognized as the most accurate and effective detection method. However, both the original radiolabeled nucleoside incorporation method and the subsequent improvement of the BrdU method based on antibody detection have their own limitations.

EdU (5-Ethynyl-2'-deoxyuridine, 5-ethynyl-2'-deoxyuridine) is a thymidine analogue containing an acetylene group, when injected into animals or incubating cells cultured in vitro, these small molecules can quickly diffuse to various organs and tissues, and infiltrate into the cells, and can replace thymidine (T) into newly synthesized DNA during cell proliferation. The acetylene group in the EdU molecule can react with the fluorescently iF488 labeled azide compound probe to form a stable triazole ring under the catalysis of copper ions, so the newly synthesized DNA can be labeled with the corresponding fluorescent probe. Compared with the radiolabeled nucleoside incorporation method, the EdU detection method has no limiting factors such as radioactive contamination; compared with the BrdU detection method, the EdU detection method does not require DNA denaturation or antigen-antibody reaction, which greatly reduces the complexity of the experiment and also make the experiment more time-saving, more sensitive, more stable and more accurate.

This kit can be used to detect cell proliferation in cultured cells or animal tissues. The fluorescent probe in this kit is pink (Far Infrared) fluorescence, the maximum excitation wavelength is 656 nm, and the maximum emission wavelength is 670 nm. After the proliferating cells are labeled, the cell nucleus will show bright pink (Far Infrared) fluorescence, and the cell nucleus will be jointly labeled with the matching conventional nuclear dye (This kit provides Hoechst 33342 cell nuclear dye), you can use fluorescence microscope, laser confocal microscope and other instruments to directly observe cell proliferation; you can also use flow cytometry to detect the fluorescence intensity of cultured cells in vitro, and then determine the cell cycle based on the fluorescence intensity DNA replication activity in mid-S phase.

Storage and Shipping Conditions

Ship with wet ice; store at -20°C in the dark. Catalyst (Reagent A) and Reaction Buffer can be stored at 4°C; Valid for 12 months.

Product Contents

Component Number	Component	G1604-100T

G1604-1	EdU Stock Solution (10 mM)	100 μL
G1604-2	Catalyst (Reagent A)	120 μL
G1604-3	Fluorescent Stain iF647 (Reagent B)	50 μL
G1604-4	Catalytic Additive (Reagent C)	2×100 mg (powder)
G1604-5	Reaction Buffer	20 mL
G1604-6	Hoechst 33342 Stain	30 μL
Manual		One copy

Note: The above reaction times are corresponding to 96-well plate detection.

Before starting

1. Serum-containing cell culture medium;
2. Permeabilization buffer: buffer containing 0.2-0.5% Triton X-100 (recommended G1204);
3. Fixative: 4% paraformaldehyde (recommended G1101) or other similar reagents;
4. PBS buffer (recommended G4202);
5. Ultrapure water;
6. Animal modeling and tissue section related reagents (animal tissue cell proliferation detection).

Assay Protocol / Procedures

1. Pretreatment of cultured cells in vitro:

1.1. Plant the cells evenly in the cell culture plate at a certain density (the planting density is determined by factors such as cell size, growth speed, etc.). After the cells adhere to the wall or return to a normal state, perform corresponding drug stimulation and other treatments. (For suspension cells, please follow the normal operation method of suspension cells. The whole experiment needs to add centrifugation and other steps).

1.2. The catalytic additive (Reagent C) was centrifuged at low speed, 100 mg was taken and dissolved by adding 1 mL of ultrapure water and dispensed and stored at -20°C, the remaining powder was kept as reserve.

2. In vitro cellular EdU labeling, fixation and permeabilization:

2.1. Prepare **2×EdU incubation working solution:** add 2 μL **EdU storage solution (10 mM)** to every 1 mL of complete cell culture medium, which is 20 μM **2×EdU incubation working solution**, and put it in the incubator to preheat (the recommend EdU working concentration is 10 μM for preliminary experiments);

2.2. In the half-changing mode, aspirate half of the original cell culture medium in the culture plate, and add an equal volume of preheated **2×EdU incubation working solution**, and incubate for a certain period of time (the duration of the incubation generally depends on the growth cycle of the corresponding cells, which usually accounts for about 10% of the cell cycle. For mostly adherent and fast-growing cells, incubation for about 2 h is recommended. For specific cases, it needs to be adjusted with the cell characteristics, the actual situation after treatment, etc. If a longer incubation time is required, the EdU working concentration can be appropriately reduced; for a shorter time, the EdU concentration can be appropriately increased);

2.3. After EdU incubation, wash with PBS buffer for 1-2 times, add fixing fluid to cover the cells, and fix at room temperature for 15 minutes (if flow cytometry is required, the adherent cells should

be digested and resuspended before this step fix, follow the suspension cell processing method); Wash 2-3 times with PBS buffer, 3-5 min each time;

2.4. Remove the PBS buffer, add permeabilization solution to cover the cells, and incubate at room temperature for 15 minutes;

2.5. Remove the permeabilization solution, wash 1-2 times with PBS buffer, 3-5 min each time. Then go to step 4.

3. Animals EdU injection modeling as well as tissue section processing:

3.1. According to experimental requirements, one or more EdU injections are used to model animals by intraperitoneal injection, intramuscular injection, subcutaneous injection, tail vein injection, etc. Generally, the ratio of EdU dosage to animal body weight is 5 mg/kg, the actual injection dose depends on the research content and animal conditions. The EdU storage solution provided in this kit is mainly used for in vitro cell EdU labeling. If you need to model an animal with EdU, you can order the EdU reagent separately (Cat. No.: G5059);

3.2. Epithelial cells such as the small intestine proliferate quickly, while brain cells proliferate slowly. The faster-growing tissues usually take less than 12 hours for labeling, while those slower-growing tissues may take several days for labeling.The optimal labeling time was determined according to the specific experiment. Due to the rapid proliferation of intestinal epithelial tissue, such tissue was recommended as a reference for labeling.

3.3. After the animal is killed according to the specified standards, the tissues needed are taken out and frozen sections or paraffin sections are made according to the conventional procedures:

a. **For frozen sections:** return the sections to room temperature, add an appropriate amount of Fixing fluid, and fix at room temperature for 15 minutes. Remove the Fixing fluid and wash 3 times with PBS buffer for 3-5 min each; remove the PBS buffer and cover the tissue with an appropriate amount of permeabilization solution and incubate at room temperature for 10-15 min; remove the permeabilization solution and wash with PBS buffer 1- 2 times, 3-5 min each time. Then go to step 4.

b. **For paraffin sections:** Deparaffinize and rehydrate the sections, and wash with PBS for 5 min. Remove the PBS buffer, add permeabilization solution to cover the cells or tissues, and incubate at room temperature for 15 min; Then wash with PBS buffer for 1-2 times, each time for 3-5 min. Then go to step 4.

4. EdU click reaction:

4.1. During cell or tissue fixation and perforation, preparation of reaction solution: mix the reagents according to the following ratio, the volume of preparation can be increased or decreased in proportion to the number of samples.

Component	Volume (for cell)	Volume (for Histological section)
reaction buffer	935 μL	928 μL
Reagent A	10 μL	10 μL
Reagent B (iF647 dye)	5 μL	12 μL
Reagent C	50 μL	50 μL
total capacity	**1000 μL**	**1000 μL**

4.2. Remove the PBS buffer from the cells or sections, add the reaction solution, shake gently to ensure that the reaction solution covers all the cells or tissues, and incubate for 30 min at room temperature in the dark;

4.3. Remove the reaction solution, wash 2-3 times with PBS buffer, 3-5 min each time (If there is no other special requirement, the fluorescence intensity can be detected by flow cytometry or the

fluorescence effect can be detected by other instruments).

5. **Nuclear stain(optional):**

   5.1. Dilute Hoechst 33342 staining solution with PBS buffer at a ratio of 1:500-1000, add to the sample to cover the cells, and incubate for 5 min;

   5.2. Remove Hoechst 33342 staining solution, wash 2-3 times with PBS buffer, 3-5 min each time.

6. **Imaging and detection analysis:**

   Use fluorescence microscope or confocal microscope to detect processed cells or tissue section samples, and analyze the proportion of proliferating cells. Alternatively, cells cultured in vitro can be collected and the fluorescence intensity can be measured by flow cytometry (it is recommended to use cell samples not labeled with EdU as a negative control for the flow cytometry assay and to choose the appropriate voltage), and based on the fluorescence intensity, the DNA replication activity of the S-phase in the cell cycle can be determined. The fluorescent dye iF647 (Reagent B) in this kit corresponds to the spectral characterization of Ex/Em: 656 nm/670 nm (pink); Hoechst 33342 staining solution corresponds to the spectral characterization of Ex/Em: 346 nm/460 nm (blue).

Note:

1. For cultured cells, the specific EdU concentration and incubation time can be adjusted appropriately depending on the sample and research purpose.
2. Some tissue cells proliferate slowly. In order to avoid poor modeling effect, it is recommended to select tissue samples with fast proliferation as reference samples (such as intestinal tissue).
3. If the background color is too dark, it may be caused by insufficient washing, a long time fixed, and residual fixative, etc.in the experiment.
4. Reagent C (EdU catalytic addition reagent) is easy to oxidize. Try to avoid prolonged exposure to the air. After being prepared as an aqueous solution, it is recommended to store in aliquot; Tested, such as EdU catalytic additive reagent color changes slightly, click reaction catalytic system is still able to proceed normally. if reagent C appears brown, it indicates that the component has expired, so please discard it.
5. For your health and safety, please wear lab coats and gloves during operation.

Servicebio® Calcein AM Cell Viability Assay Kit（CCK-F）

Cat. #：G1609

Product Information

Product Name	Cat. No	Spec.
Calcein AM Cell Viability Assay Kit（CCK-F）	G1609-100T	100T
	G1609-500T	500T

Product Description/Introduction

Calcein AM is based on Calcein (Calcein Acetoxymethyl Ester), and introduces an acetylmethoxymethyl ester (AM) group, which not only masks the molecular portion of Calcein that chelates calcium but also enhances its hydrophobicity, thus allowing Calcein AM to easily penetrate into the membrane of living cells, and to be sheared by endogenous cellular esterases to Calcein. Calcein, which has lost its AM group, cannot easily pass through the cell membrane and is thus retained inside the cell; in addition, the molecular part of the chelated calcium is partially exposed, allowing the Calcein probe to bind to calcium ions in the living cell and emit a strong green fluorescence. However, dead cells lack esterase and cannot be hydrolyzed to Calcein AM, so it cannot label dead cells. Calcein AM has low cytotoxicity and has little effect on cellular functions, such as cell proliferation or lymphocyte chemotaxis, making it an excellent fluorescent probe for staining live cells.

This product, Calcein AM Cell Activity Assay Kit, also known as Cell Fluorescence Counting Kit (CCK-F), is an assay kit to evaluate the activity, toxicity, and proliferation of cells by labeling live cells with Calcein AM fluorescent probe. This kit has been optimized and tuned, not only has high detection sensitivity and wide linear range, but also requires only a short incubation time to complete the detection of cells. It is easy to operate and does not require radioisotope labeling or steps such as crystallization and dissolution, which can reduce the errors caused by experimental operations and improve the accuracy and reproducibility.

Storage and Shipping Conditions

Ship with wet ice; store at -20°C in the dark.; Try to avoid repeated freezing and thawing, valid for 12 months.

Product Contents

Component Number	Component	G1609-100T	G1609-500T
G1609-1	Calcein AM Solution	100 μL	500 μL
G1609-2	CCK-F Assay Buffer	10 mL	50 mL
Manual		One copy	

Assay Protocol / Procedures

1. Preparation of Calcein AM working solution

For a 96-well cell culture plate, use 100μl of calcein AM working solution per well. Prepare an appropriate amount of calcein AM working solution as indicated in the following table and mix thoroughly.

Component	10 assays	50 assays	100 assays
Calcein AM Solution	10 μL	50 μL	100 μL
CCK-F Assay Buffer	1 mL	5 mL	10 mL

2. Adherent cell detection:

The following steps correspon to the 96-well plate detection scheme, and other muti-well plates systems need to be adjusted according to the situation.

a. Cell seeding: cells are evenly seeded in a 96-well plate at a certain density, and treated with drugs or other pre-treatments (the seeding density is determined by factors such as cell size, growth rate, etc.);

b. (Optional) Cell washing: Remove the culture medium and wash cells once to twice with PBS (recommended G4202) to remove serum, phenol red and/or drugs that interfere with the assay.

c. Probe labeling: After removal of cell culture medium or PBS buffer, add 100 μL calcein AM working solution and incubate at 37°C for 30 min in the dark.

d. (Optional) Cell incubation: Continue to incubate for 15-30 min with the new cell culture medium to ensure that the intracellular Calcein AM is fully hydrolyzed.

e. Fluorescence detection: After incubation, measure fluorescence with a fluorescence microplate reader (the maximum excitation wavelength of Calcein AM probe is 501 nm, and the maximum emission wavelength is 521 nm.) and calculate the cell viability. The fluorescence intensity is proportional to the number of viable cells. Fluorescence microscopy can also be used to observe the staining results.

3. Suspension cell assay:

a. Cell seeding: cells are evenly seeded in a 24-well plate at a certain density, and treated with drugs or other pre-treatments (the seeding density is determined by factors such as cell size, growth speed, etc.);

b. (Optional) Cell washing: Centrifuge cell culture at 1,000×g for 3-5 minutes, remove the supernatant and wash cells twice with PBS to remove serum, phenol red and/or drugs that interfere with the assay.

c. Probe labeling: After centrifuging at 1000 g for 3-5 min, remove the cell culture medium or PBS buffer, add an appropriate amount of Calcein AM working solution, and incubate at 37°C for 30 min in the dark;

d. (Optional) Cell incubation: Continue to incubate for 15-30 min with the new cell culture medium to ensure that the intracellular Calcein AM is fully hydrolyzed.

e. Fluorescence detection: After incubation, measure fluorescence with a fluorescence microplate reader or flow cytometry (the maximum excitation wavelength of Calcein AM probe is 501 nm, and the maximum emission wavelength is 521 nm.). Cell viability is proportional to fluorescence intensity. Depending on the purpose of the experiment, nuclei can also be further counterstained or detected with other instruments such as fluorescence microscopy.

Note

1. Briefly centrifuge Calcein AM solution before use to reduce reagent loss.
2. Calcein AM decomposes easily in humid environments. Aliquot upon receipt, seal tightly, and store at -20℃.
3. Calcein AM is unstable in aqueous solutions. The Calcein AM working solution is ready-to-use and valid for use within 24 hours.
4. The optimal incubation time is different for different cell types, which can be adjusted and optimized based on the staining results.
5. Use a black multi-well plate to grow cells when fluorescence microplate reader is used for the measurement.
6. Fluorescent dyes are subject to quenching, handle and store away from light.
7. For your safty and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® Calcein AM Cell Viability Assay Kit（CCK-F）

Cat. #：G1609

Product Information

Product Name	Cat. No	Spec.
Calcein AM Cell Viability Assay Kit（CCK-F）	G1609-100T	100T
	G1609-500T	500T

Product Description/Introduction

Calcein AM is based on Calcein (Calcein Acetoxymethyl Ester), and introduces an acetylmethoxymethyl ester (AM) group, which not only masks the molecular portion of Calcein that chelates calcium but also enhances its hydrophobicity, thus allowing Calcein AM to easily penetrate into the membrane of living cells, and to be sheared by endogenous cellular esterases to Calcein. Calcein, which has lost its AM group, cannot easily pass through the cell membrane and is thus retained inside the cell; in addition, the molecular part of the chelated calcium is partially exposed, allowing the Calcein probe to bind to calcium ions in the living cell and emit a strong green fluorescence. However, dead cells lack esterase and cannot be hydrolyzed to Calcein AM, so it cannot label dead cells. Calcein AM has low cytotoxicity and has little effect on cellular functions, such as cell proliferation or lymphocyte chemotaxis, making it an excellent fluorescent probe for staining live cells.

This product, Calcein AM Cell Activity Assay Kit, also known as Cell Fluorescence Counting Kit (CCK-F), is an assay kit to evaluate the activity, toxicity, and proliferation of cells by labeling live cells with Calcein AM fluorescent probe. This kit has been optimized and tuned, not only has high detection sensitivity and wide linear range, but also requires only a short incubation time to complete the detection of cells. It is easy to operate and does not require radioisotope labeling or steps such as crystallization and dissolution, which can reduce the errors caused by experimental operations and improve the accuracy and reproducibility.

Storage and Shipping Conditions

Ship with wet ice; store at -20°C in the dark.; Try to avoid repeated freezing and thawing, valid for 12 months.

Product Contents

Component Number	Component	G1609-100T	G1609-500T
G1609-1	Calcein AM Solution	100 μL	500 μL
G1609-2	CCK-F Assay Buffer	10 mL	50 mL
Manual		One copy	

Assay Protocol / Procedures

1. Preparation of Calcein AM working solution

For a 96-well cell culture plate, use 100μl of calcein AM working solution per well. Prepare an appropriate amount of calcein AM working solution as indicated in the following table and mix thoroughly.

Component	10 assays	50 assays	100 assays
Calcein AM Solution	10 μL	50 μL	100 μL
CCK-F Assay Buffer	1 mL	5 mL	10 mL

2. Adherent cell detection:

The following steps correspon to the 96-well plate detection scheme, and other muti-well plates systems need to be adjusted according to the situation.

a. Cell seeding: cells are evenly seeded in a 96-well plate at a certain density, and treated with drugs or other pre-treatments (the seeding density is determined by factors such as cell size, growth rate, etc.);

b. (Optional) Cell washing: Remove the culture medium and wash cells once to twice with PBS (recommended G4202) to remove serum, phenol red and/or drugs that interfere with the assay.

c. Probe labeling: After removal of cell culture medium or PBS buffer, add 100 μL calcein AM working solution and incubate at 37°C for 30 min in the dark.

d. (Optional) Cell incubation: Continue to incubate for 15-30 min with the new cell culture medium to ensure that the intracellular Calcein AM is fully hydrolyzed.

e. Fluorescence detection: After incubation, measure fluorescence with a fluorescence microplate reader (the maximum excitation wavelength of Calcein AM probe is 501 nm, and the maximum emission wavelength is 521 nm.) and calculate the cell viability. The fluorescence intensity is proportional to the number of viable cells. Fluorescence microscopy can also be used to observe the staining results.

3. Suspension cell assay:

a. Cell seeding: cells are evenly seeded in a 24-well plate at a certain density, and treated with drugs or other pre-treatments (the seeding density is determined by factors such as cell size, growth speed, etc.);

b. (Optional) Cell washing: Centrifuge cell culture at 1,000×g for 3-5 minutes, remove the supernatant and wash cells twice with PBS to remove serum, phenol red and/or drugs that interfere with the assay.

c. Probe labeling: After centrifuging at 1000 g for 3-5 min, remove the cell culture medium or PBS buffer, add an appropriate amount of Calcein AM working solution, and incubate at 37°C for 30 min in the dark;

d. (Optional) Cell incubation: Continue to incubate for 15-30 min with the new cell culture medium to ensure that the intracellular Calcein AM is fully hydrolyzed.

e. Fluorescence detection: After incubation, measure fluorescence with a fluorescence microplate reader or flow cytometry (the maximum excitation wavelength of Calcein AM probe is 501 nm, and the maximum emission wavelength is 521 nm.). Cell viability is proportional to fluorescence intensity. Depending on the purpose of the experiment, nuclei can also be further counterstained or detected with other instruments such as fluorescence microscopy.

Note

1. Briefly centrifuge Calcein AM solution before use to reduce reagent loss.
2. Calcein AM decomposes easily in humid environments. Aliquot upon receipt, seal tightly, and store at -20℃.
3. Calcein AM is unstable in aqueous solutions. The Calcein AM working solution is ready-to-use and valid for use within 24 hours.
4. The optimal incubation time is different for different cell types, which can be adjusted and optimized based on the staining results.
5. Use a black multi-well plate to grow cells when fluorescence microplate reader is used for the measurement.
6. Fluorescent dyes are subject to quenching, handle and store away from light.

Servicebio® LDH Cytotoxicity Assay Kit

Cat. #：G1610-100T

Product Information

Product Name	Cat. No	Spec.
LDH Cytotoxicity Assay Kit	G1610-100T	100T

Product Description/Introduction

Lactate dehydrogenase (LDH) is a terminal enzyme of the glycolytic pathway, widely found in animals, plants, and microorganisms, and is involved in catalyzing the reversible reaction between pyruvate and lactate. Normally LDH is abundant in the cytoplasm of cells, and normal cells cannot pass intracellular LDH freely through the cell membrane due to the barrier protection of the cell membrane. However, when cells are damaged or die, the barrier protection of the cell membrane disappears and intracellular LDH is released into the culture medium. Cytotoxicity can be quantified by detecting the amount of LDH released into the culture medium by cell membrane rupture.

The main principle of Lactate Dehydrogenase (LDH) Cytotoxicity Detection Kit is to use LDH to reduce NAD^+ to NADH, and further NADH and INT (tetrazolium salt) catalyzed by Diaphorase (Lipoic Acid Dehydrogenase) to produce NAD^+ and red filth, the amount of filth produced and the amount of LDH is a linear correlation, and is able to produce an absorption peak at 490 nm. Absorption peak at 490 nm, so it can be quantitatively analyzed by the instrument. This kit is mainly used for cytotoxicity assays based on LDH release as an indicator, but can also be used for cell proliferation and toxicity assays based on total cellular LDH.

Storage and Shipping Conditions

Ship with wet ice; Store at -20℃ for up to 12 months. Avoid repeated freeze-thaws of Enzyme Solution. INT Solution should be protected from light.

Product Contents

Component Number	Component	G1610-100T
G1610-1	10× Cell Lysis Buffer	1.5 mL
G1610-2	Reaction Substrate	2×1 mL
G1610-3	INT Solution	200 μL
G1610-4	Enzyme Solution	2×150 μL
G1610-5	Reaction Buffer	10 mL
Manual		One copy

Assay Protocol / Procedures

1. Sample Preparation:

1) Detection of extracellular LDH

a. Cell seeding: Seed cells in 96-well cell culture plate for appropriate time depending on the type of cells. The confluence of cells to be tested should exceed 80-90%. And multiple controls can be set according to experimental requirements.

background control: contains culture medium only and no cells.

sample control: contains culture medium and cells. Untreated cell wells.

Sample maximum enzyme activity control: contains culture medium and cells. Detection of total intracellular LDH after lysis of untreated cells

Treated Sample (experimental group): According to the desired method, treat cells with appropriate drug at different concentrations.

b. Cell treatment: Remove the culture medium and wash cells once with PBS solution (recommended G4202). Add fresh culture medium (culture medium containing low serum, serum-free or drug-containing culture medium) into each well, incubate for a certain period.

c. Sample collection: Centrifuge the cell culture plate at 1,000 × g for 5 min. Transfer 80 μL of supernatant from each well into a new 96-well plate, after which the samples were assayed sample preparation for the control well with the maximum enzyme activity of the sample, please Refer to the sample preparation steps for total intracellular LDH assay).

2) Detection of total intracellular LDH

a. Cell seeding: Cells are inoculated in 96-well cell culture plates at a suitable density, and multiple subgroups can be set up according to experimental needs;

b. Cell treatment: according to the experimental design, use drug-containing or drug-free medium (may contain serum) to incubate cells;

c. Cell lysis: After reaching the predetermined time, remove the original medium, wash it with PBS once, add 120 μL of cell lysis working solution (10× cell lysis solution diluted 10 times with PBS), and incubate in the incubator for 30-60 min ;

d. Sample collection: Centrifuge the cell culture plate at 1,000× g for 5 min, and add 80 μL of the lysis supernatant to a new 96-well plate, followed by assay.

3) (Optional) LDH standard sample preparation

If absolute quantification of LDH enzyme activity is desired, purchase LDH standard individually and prepare LDH standards at different concentrations: 10 mU/mL, 5 mU/mL, 2.5mU/mL, 1.25 mU/mL, 0.65 mU/mL and 0 mU/mL. At 80 μL per well, the gradient was added to the 96-well plate.

2. LDH Detection:

1) According to the number of samples to be tested, refer to the table below to prepare an appropriate amount of LDH detection working solution.

Note: it corresponds to 96-well plate, and for other specifications of well plates can be adjusted as needed);

	1 Sample	10 Samples	20 Samples	50 Samples
Reaction Substrate	20 μL	200 μL	400 μL	1 mL
INT Solution	2 μL	20 μL	40 μL	100 μL
Enzyme Solution	3 μL	30 μL	60 μL	150 μL
Reaction Buffer	55 μL	550 μL	1.1 mL	2.75 mL
Total Volume	80 μL	800 μL	1.6 mL	4 mL

2) Add 80 μL LDH working solution into 80μL supernatant in 96-well plate prepared in step 1 (V_{sample}: $V_{LDH\ detection\ working\ solution}$ = 1:1), mix by gentle tapping.

3) The cells were incubated in a cell culture incubator, protected from light for 30 min, or wrapped in tin foil and incubated on a horizontal shaker for 30 min at room temperature;

4) Measure the absorbance of all controls and samples with a plate reader at 490nm. The reference wavelength should be 600nm or greater.

3. LDH Result Calculation:

1) Routine LDH (release) cytotoxicity assay:

a. Subtract the OD490 of the background control from the OD490 of the sample control, sample maximum enzyme activity control, treated sample wells, etc., and use them for subsequent calculations.

b. Calculation of cytotoxicity or death rate (%) = (OD490 of treated sample - OD490 of sample control) / (OD490 of maximum enzyme activity of sample control - OD490 of sample control) × 100%

2) Relative quantification of LDH enzyme activity (according to the calculation results, it is possible to compare whether there are statistical differences between different sample treatment groups, etc.):

a. Determine the OD490 of a known concentration of LDH standard;

b. LDH activity of the sample to be tested (mU/mL) = (OD490 of sample well - OD490 of background blank control well) / (OD490 of standard product - OD490 of background blank control well) × standard concentration (mU/mL)

3) Absolute quantification of LDH enzymatic activity:

a. Measure the OD490 of a series of LDH gradient standard products, draw a standard curve according to the obtained absorbance value, and calculate the trend formula: Y(OD490) = A×LDH activity unit (mU)+B, the trend can be calculated by software such as Excel the slope and intercept of the line;

b. LDH activity in the detection system (mU) = (sample well OD490-background blank control well OD490-B)/A

c. Sample LDH activity (mU/mL) = LDH activity in the detection system (mU) / detection volume (mL)

Note

1. The density of the cells should not exceed 85% or more, factors such as the state of the cells and the density of the cells will have some effect on the cellular LDH release, and the samples are prepared, try to complete the test on the same day, do not freeze.
2. Serum contains lactate dehydrogenase, it is recommended to use serum-free or low serum medium, if you have to use 10% serum, please set up a cell-free control group to eliminate background interference.
3. The operation is as gentle as possible and bubbles are to be avoided so as not to affect the experimental results.
4. For your safty and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® ATP Luminescence Cell Viability Assay Kit

Cat. No.: G1612

Product Information

Product Name	Cat. No.	Spec.
ATP Luminescence Cell Viability Assay Kit	G1612-100T	10 mL
	G1612-1000T	100 mL

Product Description/Introduction

ATP is an important energy molecule in the cell which plays an important role in various physiological and biochemical activities of the cell, and is known as the energy currency in the organism. The cellular state and cell numbers show a correlation with ATP. Therefore, cell number or viability can be assessed by the relative level of ATP.

This kit is based on the principle of ATP-dependent luciferase catalyzing luciferin to produce fluorescence, which can effectively detect and evaluate the intracellular ATP level. Compared with the traditional methods such as CCK-8 and MTT, fluorescence method has the advantages of high sensitivity and wide range.

Storage and Shipping Conditions

Ship with dry ice; Store at -80℃ away from light for 12 months; Store at -20°C away from light, recommended for use within 6 months.

Product Content

Component	G1612-100T	G1612-1000T
ATP Luminescence Cell Viability Assay Kit	10 mL	100 mL
Manual	1 pc	

Assay Protocol / Procedures

1. **Cell pre-processing work:** Inoculate the cells at a certain density in a 96-well plate (please choose a white opaque well plate suitable for cell culture or use a regular cell culture plate. When performing the detection, transfer the solution to the white detection wells to minimize interference between adjacent wells.) and pre-treat the cells according to the purpose of the experiment;
2. **Sample preparation for ATP standards (optional):** The ATP standard (self-contained) is gradually diluted with buffer such as PBS or basal medium and added to the well plate at 100 μL/well;
3. **Cell Viability Assay:**
   - 3.1. Take out the ATP Luminescence Cell Viability Assay Reagent in advance, thaw it and return it to room temperature;
   - 3.2. Remove the cell culture plate and equilibrate at room temperature for 5-10 min;
   - 3.3. The ATP Luminescence Cell Viability Assay Reagent is added directly to the well plate at 100 μL/well;
   - 3.4. Shake horizontally for 1-2 min or tap to mix;
   - 3.5. After standing for 3 min, the chemiluminescence assay can be performed using a Luminometer, a multifunctional enzyme labeler with a chemiluminescence detection module, or other instruments capable of detecting bioluminescence (note: the sample itself does not emit light in the fluorescence method, but needs to be excited by a specific wavelength of excitation light and then received through a special channel. In chemiluminescence, the sample itself emits light and

does not need to be excited by a specific wavelength of excitation light in order to be detected by the corresponding equipment);

4. **Data analysis:** The following data analysis is based on subtracting the background blank

4.1. Relative cell number (proliferation) analysis: According to the fluorescence intensity value, determine the number of cells.

4.2. Cell viability calculation (cell proliferation viability or cytotoxicity viability):

$$cell\ viability\ (\%) = \frac{\text{A (Dosing group fluorescence value)} - \text{A0 (Blank control group fluorescence value)}}{\text{A1 (Fluorescence value of non} - \text{dosed group)} - \text{A0 (Blank control group fluorescence value)}}$$

Note:

A (dosing group): treated cells + ATP luminescence cell viability assay reagent

A1 (no dosing group): normal untreated cells + ATP luminescence cell viability assay reagent

A0 (blank control group): cell culture medium (no cells) + ATP luminescence cell viability assay reagent

4.3. Quantitative analysis of ATP content (optional):

4.3.1. The fluorescence intensity value of the detected ATP standard curve is subtracted from the fluorescence intensity value data of the blank control group, and the standard curve is plotted using software such as Excel, and the formula is obtained:

$$\text{Y (Fluorescence intensity value)} = \text{a} \times \text{X (ATP content)} + \text{b}$$

where a represents the slope and b represents the intercept.

4.3.2. The X-value (ATP content) in the assay system is calculated according to the formula:

$$\text{X (ATP content)} = \frac{[\text{A (Dosing group fluorescence value)} - \text{A0 (Blank control group fluorescence value)}] - \text{b}}{\text{a}}$$

Calculate the value of Y by substituting the slope a and intercept b from 4.3.1 into the above equation.

4.3.3. Calculation of ATP content in samples:

$$Detection\ of\ ATP\ in\ samples = \frac{X - value\ in\ the\ detection\ system\ (ATP\ content)}{Detection\ volume} \times Sample\ dilution\ ratio$$

Note

1. To ensure that the test reagent is used effectively, it is recommended to store in small portions and avoid repeated freezing and thawing.
2. The testing time warp is controlled within 5-30 min to ensure the accuracy of the testing data.
3. Part of the reagent precipitation after thawing, is a normal phenomenon, before use fully shaken to ensure its complete dissolution.
4. Try to avoid light and add the test reagents within a short period of time. It is recommended to use a multiwell pipette to add samples and pay attention to the consistency of the pipetting volume in each well of the pipette.
5. For your health and safety, please wear lab coat and gloves during operation.

Servicebio® Cell Counting Kit-8 Plus

Cat. No.: G1613

Product Information

Product Name	Cat. No.	Spec.
Cell Counting Kit-8 Plus	G1613-1ML	1 mL
	G1613-5ML	5 mL

Product Description/Introduction

This kit uses WST-8 compound, which can be reduced by dehydrogenase to form water-soluble orange formaldehyde compound in mitochondria, and the maximum absorption peak is 450nm. The production of formaldehyde is proportional to the number of living cells, that is, the more living cells, the darker the color; The higher the cytotoxicity, the lighter the color. For the same cells, there is a linear relationship between the depth of color and the number of cells.

Cell Counting Kit-8 Plus(CCK-8 Plus), is optimized on the basis of conventional CCK-8 kits and takes only 0.5 to 1 hour to complete the detection, with faster detection, higher sensitivity, and wider linear range.

Storage and Shipping Conditions

Ship with wet ice; store at 2-8℃ in the dark for up to 12 months, and -20℃ to 24 months.

Product Contents

Product Name	G1613-1ML	G1613-5ML
Cell Counting Kit-8 Plus	1 mL	5 mL
Manual	One copy	

Assay Protocol / Procedures

Cell Proliferation-Viability-Toxicity Assay

(1) In a 96-well plate, plant a certain amount of 100 µL cell suspension and pre-cultured in an incubator (37℃, 5% CO2) for 24 hours.

(2) Add 10 µL of different concentrations of the test compounds to the culture plate. Incubate for an appropriate period of time (eg: 6, 12, 24, 48 or 72hours).

(3) Add 10 µL of CCK-8 plus solution to each well (be careful not to create air bubbles in the wells, which will affect the OD reading).

Note: If the test compounds is oxidative or reducing, the effect of the drug can be removed by replacing the medium with fresh medium before adding CCK-8.

(4) Incubate the plate in the incubator for 0.5-2 hours.

(5) Measure the absorbance at 450 nm with a microplate reader.(wavelengths greater than 600nm, such as 650nm, can be used as a reference wavelength for dual-wavelength determination).

(6) If the OD value is not to be measured temporarily, you can add 10 µL of 0.1M HCl or 1% SDS (W/V) solution to each well, and cover the culture plate and store it at room temperature in the dark. The absorbance does not change within 24 hours.

Vitality Calculation

Cell viability (%)=[A (dosed)-A (blank)]/[A (control)-A (blank)]×100%

Cytotoxic viability (%)=[A (control)-A (dosed)]/[A (control)-A (blank)]×100%

A (dosed): Absorbance of wells with cells, CCK-8 plus solution, and drug solution

A (blank): absorbance of wells with medium and CCK-8 plus solution without cells

A (control): Absorbance of wells with cells, CCK-8 plus solution and no drug solution

Note

1. This kit employs the reduction reaction catalyzed by dehydrogenase. Reducing agents or antioxidants that may interfere with the assay should be removed from samples to be tested.
2. It is recommended to perform pre-experiment to explore the number of inoculated cells and the incubation time after adding CCK-8 plus solution. Leukocytes may need to be cultured for a longer period of time.
3. If a 450 nm filter is not available, a filter with absorbance between 430-490 nm can be used, but 450 nm has the highest detection sensitivity.
4. The absorbance of phenol red in the medium can be eliminated by subtracting the absorbance of the background in the blank well during calculation, so it will not affect the detection.
5. Air bubbles in wells should be removed before the measurement of absorbance.
6. For your safty and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® Calcein-AM/PI Living / Dead cell Double Staining Kit

Cat. #：G1707-100T

Product Information

Product Name	Cat. No.	Spec.
Calcein-AM/PI Living / Dead cell Double Staining Kit	G 1707-100T	100T

Product Description/Introduction

Calcein AM is based on Calcein (Calcein acetoxymethyl ester) and introduces an acetylmethoxymethyl ester (AM) group, which not only masks the molecular portion of Calcein that chelates calcium, but also enhances its hydrophobicity, thus allowing Calcein AM to easily penetrate the membrane of living cells. When Calcein AM enters the cell, it is able to be sheared to Calcein by endogenous cellular esterases. With the loss of the AM group, Calcein cannot easily pass through the cell membrane and is thus retained inside the cell; in addition the molecular part of the chelated calcium is partially exposed and the Calcein probe is able to emit a strong green fluorescence when bound to intracellular calcium ions. Dead cells lack esterase, which cannot hydrolyze Calcein AM into Calcein, and therefore cannot be labeled.PI (propidium iodide) is able to embed itself in the cellular DNA double helix to produce red fluorescence, but it cannot directly stain the cell membranes of living cells, and can only stain dead cells where the cell membranes have been disrupted. Thus combining Calcein-AM with PI allows for staining labeling of both live and dead cells.

This kit is based on the above principles and has been optimized and debugged by our company to perform double staining labeling by Calcein-AM labeling of live cells and PI labeling of dead cells, thus allowing analysis of both live and dead cell levels.

Storage and Shipping Conditions

Ship with wet ice; Store at -20℃ away from light, valid 12 months.

Product Contents

Component Number	Component	G 1707-100T
G1707-1	Calcein-AM solution	20 μL
G1707-2	PI solution	20 μL
G1707-3	Detection buffer	10 mL
Manual		One copy

Note: The above reaction times are a single 100 μL system detection.

Assay Protocol / Procedures

1. Preparation of Calcein AM/PI working solution
   a) Remove Calcein-AM solution, PI solution and detection buffer from the freezer, then allow them to warm to room temperature.
   b) Configure the working solution according to the need, the configuration system can refer to the following table, note that different cells have different characteristics, the experiment can be added or reduced according to the actual situation of the probe concentration used, most of the cell dilution ratio between 1:500-2000;

	10 samples	50 samples	100 samples
Calcein-AM solution	2 μL	10 μL	20 μL

PI solution	2 μL	10 μL	20 μL
Detection buffer	1 mL	5 mL	10 mL

2. Cell staining marker:

a) According to experimental requirement, treat the cells with drugs or other stimulations;

b) For adherent cells, collect the supernatant first, digest remaining adherent cells by trypsin and mix all cells well. For suspended cells, collect all cells directly.

c) Centrifuge the collected cells at 800-1,000 rpm for 3-5 min, remove the supernatant, and thoroughly wash the cells 2-3 times with PBS or other buffer to remove the residual esterase activity;

d) Add calcein AM/PI detection working solution to cell pellet obtained above and resuspend cells gently and thoroughly. We recommend Calcein AM/PI detection working solution to resuspend the cells to control the cell density at 1×10^5~1×10^6 /mL.

e) Incubate at 37ºC for 15-30 min in the dark.

3. Cell staining observation:

a) According to the experimental requirements, fluorescence microscope, flow cytometry, microplate reader and other instruments can be used to observe and analyze the level of living cells and dead cells;

b) The living cells labeled by Calcein-AM are green fluorescent, with EX/EM = 494/517 nm; The labeled dead cells of PI are red fluorescence, EX/EM=535/617 nm.

Note

1. Due to different cell-to-cell properties and different detection methods, the probe concentration and incubation time range described in step are for reference only and it can be adjusted according to the specific situation.
2. All dyes have quenching problems, Please be away from light while handling fluorescent dyes or stained samples to slow down the quenching of fluorescence.
3. Calcein-AM is very sensitive to humidity, please dispense appropriately according to the experimental arrangement when using for the first time.Calcein AM/PI assay working solution should be dispensed and used now.
4. For your health and safety, please wear safety glasses, gloves, or protective clothing.

Servicebio® MTT Cell Viability Assay Kit

Cat. #：G4101

Product Information

Product Name	Cat. No	Spec.
MTT Cell Viability Assay Kit	G4101-200T	200 T
	G4101-1000T	1000 T

Product Description/Introduction

This product MTT detection kit is widely used in the determination of cell proliferation and activity, as well as the detection of drug cytotoxicity. The full name of MTT is 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide, and the Chinese name is thiazolyl blue. The redox potential in the mitochondria of living cells causes the water soluble MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to convert to an insoluble formazan product. After solubilization of the formazan with the included imethyl sulfoxide (DMSO) reagent, the concentration of the colorimetric probe is determined by an optical density measurement at 490 nm or 570 nm. Within a certain range of cells, the amount of formazan formed is proportional to the number of living cells. The number of living cells is neasured by the absorbance value (OD value). The greater of the OD value is, the more of living cells is, and the more active of the cell is (if measured for drug toxicity, the less toxic of the drug is). If using this kit to detect cell viability, the supernatant needs to be removed after the reaction, so it cannot be used for the detection of suspended cells. For detection of suspended cells, it is recommended to use products (G4103 CCK-8 kit) in our company..

Storage and Shipping Conditions

Ship with wet ice; store at 4°C away from light, valid for 3 months; or store at -20°C away from light, valid for 12 months. Avoid repeated freezing and thawing.

Product Contents

Component Number	Component	G4101-200T	G4101-1000T
G4101-1	MTT Solution	10 mL	50 mL
G4101-2	DMSO	20 mL	100 mL
Manual		One copy	

Assay Protocol / Procedures

1. After the cells adhere to the wall, dosing and transfection are carried out according to the experimental design;
2. Add 20 μL of MTT working solution to each well and incubate for 4 h in a cell incubator.
3. Carefully remove the supernatant, do not suck up the purple crystals at the bottom.

Note: After incubation, a small amount of purple crystals will appear at the bottom of the well plate, which can be observed under a 40x microscope.

4. Add 100 μL of DMSO to the well plate, incubate at 37°C for about 15 minutes (you can tap the bottom of the plate with your finger to shake slightly), until all the purple crystals are dissolved. If the purple crystals are smaller and less, the dissolution time will be shorter; if the purple crystals are larger, the dissolution time can be appropriately extended;
5. Read the absorbance at 490 nm or 570 nm. If a 570 nm filter is not available, a 560-600 nm filter can be used.

Note

1. Prior to use, precipitates that might be formed at lower temperature in DMSO should be resolubilized completely at 37℃ in a water bath.
2. This kit employs the reduction reaction catalyzed by dehydrogenase. Reducing agents or antioxidants that may interfere with the assay should be removed from samples to be tested.
3. For your safty and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® MTT Cell Viability Assay Kit

Cat. #：G4101

Product Information

Product Name	Cat. No	Spec.
MTT Cell Viability Assay Kit	G4101-200T	200 T
	G4101-1000T	1000 T

Product Description/Introduction

This product MTT detection kit is widely used in the determination of cell proliferation and activity, as well as the detection of drug cytotoxicity. The full name of MTT is 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide, and the Chinese name is thiazolyl blue. The redox potential in the mitochondria of living cells causes the water soluble MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to convert to an insoluble formazan product. After solubilization of the formazan with the included imethyl sulfoxide (DMSO) reagent, the concentration of the colorimetric probe is determined by an optical density measurement at 490 nm or 570 nm. Within a certain range of cells, the amount of formazan formed is proportional to the number of living cells. The number of living cells is neasured by the absorbance value (OD value). The greater of the OD value is, the more of living cells is, and the more active of the cell is (if measured for drug toxicity, the less toxic of the drug is). If using this kit to detect cell viability, the supernatant needs to be removed after the reaction, so it cannot be used for the detection of suspended cells. For detection of suspended cells, it is recommended to use products (G4103 CCK-8 kit) in our company..

Storage and Shipping Conditions

Ship with wet ice; store at 4°C away from light, valid for 3 months; or store at -20°C away from light, valid for 12 months. Avoid repeated freezing and thawing.

Product Contents

Component Number	Component	G4101-200T	G4101-1000T
G4101-1	MTT Solution	10 mL	50 mL
G4101-2	DMSO	20 mL	100 mL
Manual		One copy	

Assay Protocol / Procedures

1. After the cells adhere to the wall, dosing and transfection are carried out according to the experimental design;
2. Add 20 μL of MTT working solution to each well and incubate for 4 h in a cell incubator.
3. Carefully remove the supernatant, do not suck up the purple crystals at the bottom.

Note: After incubation, a small amount of purple crystals will appear at the bottom of the well plate, which can be observed under a 40x microscope.

4. Add 100 μL of DMSO to the well plate, incubate at 37°C for about 15 minutes (you can tap the bottom of the plate with your finger to shake slightly), until all the purple crystals are dissolved. If the purple crystals are smaller and less, the dissolution time will be shorter; if the purple crystals are larger, the dissolution time can be appropriately extended;
5. Read the absorbance at 490 nm or 570 nm. If a 570 nm filter is not available, a 560-600 nm filter can be used.

Note

1. Prior to use, precipitates that might be formed at lower temperature in DMSO should be resolubilized completely at 37℃ in a water bath.
2. This kit employs the reduction reaction catalyzed by dehydrogenase. Reducing agents or antioxidants that may interfere with the assay should be removed from samples to be tested.
3. For your safty and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® Brdu Detection Kit

Cat. #：G4102

Product Information

Product Name	Cat.No.	Spec.
Brdu Detection Kit	G4102-50T	50T
	G4102-100T	100T

Description/Introduction

This product BrdU detection kit is used for cell proliferation detection of cells and tissue sections. BrdU (5-bromo-2-deoxyuridine), i.e. 5-bromodeoxyuridine, is a thymidine (T) analog. The principle of this kit is that BrdU can be incorporated into DNA synthesis phase (S phase) through competition instead of thymidine t. When the cells in vigorous division are mixed with BrdU, the cells containing BrdU can be detected by using anti BrdU antibody and antibody ligase or fluorescein as the indicator system. Combined with other cell markers for double labeling, the type and proliferation rate of proliferating cells can be determined, which is of great significance to the study of cell dynamics. BrdU enters tissue cells when injected in vivo or cultured in cells. The location of the incorporated DNA is accurate, which can effectively reflect the level of newly synthesized DNA in S-phase cells. Moreover, it is less affected by the internal and external environment of the cells, and the label will not be lost.

Storage and Handling Conditions

Wet ice transport; Membrane breaking solution, blocking solution (3% BSA) and 1 M HCl can be stored at 4 ℃, 4% paraformaldehyde can be stored at room temperature, and Anti-BrdU (mouse mAb) needs to be stored at - 20 ℃, with a validity period of 12 months.

Component

Component Number	Component	G4102-50T	G4102-100T
G4102-1	Membrane breaking solution	30 mL	30 mL
G4102-2	blocking solution (3% BSA)	30 mL	30 mL
G4102-3	4% paraformaldehyde	30 mL	30 mL
G4102-4	1 M HCl	30 mL	30 mL
G4102-5	Anti-BrdU (mouse mAb)	50 uL	50 uL
Protocol		1 Pcs	

Preparation

1. Prepare PBS buffer (recommended g4202), secondary antibody, gradient ethanol, sealing agent, etc;
2. Prepare the nucleus staining solution, and g1004 hematoxylin staining solution or g1012 DAPI staining solution (ready to use type) is recommended;
3. Preparation of anti BrdU primary antibody working solution: dilute anti BrdU (mouse mAb) 100 times with blocking solution (3% BSA) to prepare anti BrdU primary antibody working solution, which is ready for use and stored at 4 ℃;
4. Secondary antibody working solution: prepare the anti mouse secondary antibody labeled with HRP

(followed by white light detection, matched with DAB color development kit, g1212 is recommended) or fluorescence (followed by fluorescence detection) according to the detection needs, and dilute it with PBS to prepare the secondary antibody working solution.

Assay Protocol / Procedures

Cell samples:

a. Cell preparation: prepare cell climbing pieces in advance to ensure normal cell state and good adhesion;

b. BrdU Incubation: BrdU containing cells are incubated in the incubator for a certain time in the absence of light. BrdU concentration and cell incubation time are different according to cell types. It is suggested to explore the best conditions in the pre experiment. The experimental conditions of the tested cells in the precautions are available for reference;

c. Cell fixation: the above cells incubated with BrdU were washed three times with PBS for 5 min each time. Add 1 ml of 4% paraformaldehyde to the well plate to cover the cells, and fix at room temperature for 20-30 min. Wash 3 times with PBS for 5 min each time;

d. Permeabilization: add membrane breaking solution to the well plate to cover the cells for 8-10 min, and wash with PBS for 3 times for 5 min each time;

e. Nuclear staining (optional):

In case of subsequent white light detection, the nuclei were stained with hematoxylin: add hematoxylin dye solution to the well plate for 5 min at room temperature, suck and discard hematoxylin dye solution, and wash with PBS for 3 times for 5 min each time;

In case of subsequent fluorescence detection, the nuclei were stained with DAPI: add DAPI dye solution to the well plate for 5 min at room temperature, suck and discard DAPI dye solution, and wash with PBS three times for 5 min each time;

f. Re fixation: add 4% paraformaldehyde dropwise to the well plate, incubate at room temperature for 10 min, and wash with PBS three times for 5 min each time;

g. Acidification: add 1 M HCl to the well plate to cover the cells, treat them at 37 ℃ for 10 min, and wash them with PBS three times for 5 min each time;

h. Post fixation: add 4% paraformaldehyde to the well plate again, incubate for 10 min at room temperature, and wash with PBS three times for 5 min each time;

i. Blocking: add blocking solution (3% BSA) to the well plate and incubate at room temperature for 30 min. Suck and discard the sealing liquid, and do not clean it;

j. Anti-BrdU antibody incubation: add anti BrdU primary antibody working solution to the well plate to cover the cells, and incubate overnight at 4 ℃. Suck and discard the anti BrdU primary antibody working solution, and wash it with PBS for 3 times for 5 min each time;

k. Secondary antibody incubation: add secondary antibody working solution to the well plate to cover the cells, and incubate at room temperature for 1H. Suck and discard the secondary antibody working solution and wash it with PBS for 3 times for 5 min each time. Note that if the fluorescent labeled secondary antibody is used, it should be protected from light during incubation and washing;

l. Seal and mirror inspection:

For HRP labeled secondary antibody, use DAB color reagent (recommended g1212) to develop the color of the cell sample, dehydrate and make it transparent. Gently pick up the climbing piece with pointed tweezers, buckle it upside down on the clean slide and seal it for microscope observation;

For the fluorescent labeled secondary antibody, add anti fluorescent quenching sealing agent (recommended g1401) onto the clean slide, gently lift the slide with pointed tweezers, and then buckle it

onto the slide for sealing, and observe with fluorescence microscope;

Tissue section samples:

a. Animal preparation: experimental animals shall be prepared in advance and BrdU labeling in vivo shall be carried out. Please refer to GC310002-100mg. After in vivo labeling, materials were taken, fixed and paraffin sections were prepared according to the experimental needs;

b. Tissue paraffin sections were dewaxed and rehydrated;

c. Repair: circle the tissue with group strokes, immerse the sections in the antigen repair solution, and heat them with microwave. Reference repair conditions: Granz microwave oven model P70D20TL-P4 (rated input power: 1180W; microwave output power: 700W), using 1 × Tris-EDTA antigen repair solution (pH 8.0, ready-to-use) (Item No. G1207), medium heat for 8min, cease heat for 8min, and turn to medium-low heat for 7min. the purpose of microwave repair is to keep the slices boiling for 15 minutes in the antigen repair solution. The purpose of microwave repair is to keep the slices boiling in the antigen repair solution for 15 minutes. Since the power of microwave ovens of different manufacturers is not the same, the time to reach boiling is not the same, and the specific repair conditions can be optimized according to the difference in power of microwave ovens. Natural cooling;

d. Nuclear staining (optional):

In case of subsequent white light detection, the nuclei were stained with hematoxylin: the sections were stained with hematoxylin at room temperature for 5 min, and washed with PBS for 3 times for 5 min each time;

In case of subsequent fluorescence detection, nuclei were stained with DAPI: DAPI dye solution was added dropwise to the tissue for 5 min at room temperature, DAPI dye solution was poured out, and sections were washed with PBS for 3 times for 5 min each time;

e. Re fixation: 4% paraformaldehyde was added dropwise to the tissue, incubated for 10 min at room temperature, and washed three times with PBS for 5 min each time;

f. Acidification: 1 M HCl was added dropwise to the sections to cover the tissues, treated at 37 ℃ for 10 min, and washed three times with PBS for 5 min each time;

g. Post fixation: add 4% paraformaldehyde to the sections again, cover the tissues, incubate them for 10 min at room temperature, and wash them with PBS for 3 times for 5 min each time;

h. Endogenous peroxidase quenching (optional): add 3% H2O2 to the sections and incubate at room temperature for 25-30min to remove endogenous peroxidase. Subsequently, the sections were washed three times with PBS for 5 min each time;

i. Blocking: add blocking solution (3% BSA) to the sections to cover the tissues, and incubate at room temperature for 30 min. Remove the sealing liquid and do not clean it;

j. Anti- BrdU antibody incubation: add anti BrdU primary antibody working solution to the sections to cover the tissues, and incubate at 4 ℃ overnight. The anti BrdU primary antibody working solution was removed and washed three times with PBS for 5 min each time;

k. Secondary antibody incubation: add secondary antibody working solution to the sections to cover the tissues, and incubate at room temperature for 1H. Remove the secondary antibody working solution and wash it three times with PBS for 5 min each time. Note that if the fluorescent labeled secondary antibody is used, it should be protected from light during incubation and washing;

l. Seal and mirror inspection:

If HRP labeled secondary antibody is used, DAB color reagent (recommended g1212) is used for color development of tissue sections, dehydration, transparency, and microscopic observation after sealing;

For fluorescent labeled secondary antibody, add anti fluorescence quenching sealing agent (recommended

g1401) dropwise and observe with fluorescence microscope after sealing.

Result Observation

When detected with HRP labeled secondary antibodies, the nuclei are dark blue and the proliferating cells are brown. If it is a fluorescent labeled secondary antibody, the nucleus shows blue fluorescence (ex = 358 nm, EM = 461 nm), and the proliferating cell is the fluorescence of the corresponding secondary antibody.

Note:

1. For cell climbing samples, the concentration and treatment time of BrdU were related to the cell type. Generally speaking, the concentration and time required to treat tumor cells are low. Cells with slow proliferation such as fibroblasts or epithelial cells need to increase the concentration and prolong the action time. The recommended concentration range is 20-100 μ M. The action time is 40 min-4 h, which can be adjusted according to the type of specific cells.

The following table shows the commonly used cell treatment concentration and time for testing, for reference only.

Cell	BrdU concentration（μM）	Incubation time（min）
A549	40	40
Hela	40	40
NRK-52e	40	40
SKOV3	80	120
H9C2	40	40

2. In order to ensure the best experimental effect, it is recommended to use other matching reagents produced by our company: hematoxylin dye solution (G1004); DAPI dye solution (G1012); DAB color development kit (G1212), anti fluorescence quenching sealing agent (G1401);

3. For your safety and health, please wear lab clothes and disposable gloves.

Servicebio® Brdu Detection Kit

Cat. #：G4102

Product Information

Product Name	Cat.No.	Spec.
Brdu Detection Kit	G4102-50T	50T
	G4102-100T	100T

Description/Introduction

This product BrdU detection kit is used for cell proliferation detection of cells and tissue sections. BrdU (5-bromo-2-deoxyuridine), i.e. 5-bromodeoxyuridine, is a thymidine (T) analog. The principle of this kit is that BrdU can be incorporated into DNA synthesis phase (S phase) through competition instead of thymidine t. When the cells in vigorous division are mixed with BrdU, the cells containing BrdU can be detected by using anti BrdU antibody and antibody ligase or fluorescein as the indicator system. Combined with other cell markers for double labeling, the type and proliferation rate of proliferating cells can be determined, which is of great significance to the study of cell dynamics. BrdU enters tissue cells when injected in vivo or cultured in cells. The location of the incorporated DNA is accurate, which can effectively reflect the level of newly synthesized DNA in S-phase cells. Moreover, it is less affected by the internal and external environment of the cells, and the label will not be lost.

Storage and Handling Conditions

Wet ice transport; Membrane breaking solution, blocking solution (3% BSA) and 1 M HCl can be stored at 4 ℃, 4% paraformaldehyde can be stored at room temperature, and Anti-BrdU (mouse mAb) needs to be stored at - 20 ℃, with a validity period of 12 months.

Component

Component Number	Component	G4102-50T	G4102-100T
G4102-1	Membrane breaking solution	30 mL	30 mL
G4102-2	blocking solution (3% BSA)	30 mL	30 mL
G4102-3	4% paraformaldehyde	30 mL	30 mL
G4102-4	1 M HCl	30 mL	30 mL
G4102-5	Anti-BrdU (mouse mAb)	50 uL	50 uL
Protocol		1 Pcs	

Preparation

1. Prepare PBS buffer (recommended g4202), secondary antibody, gradient ethanol, sealing agent, etc;
2. Prepare the nucleus staining solution, and g1004 hematoxylin staining solution or g1012 DAPI staining solution (ready to use type) is recommended;
3. Preparation of anti BrdU primary antibody working solution: dilute anti BrdU (mouse mAb) 100 times with blocking solution (3% BSA) to prepare anti BrdU primary antibody working solution, which is ready for use and stored at 4 ℃;
4. Secondary antibody working solution: prepare the anti mouse secondary antibody labeled with HRP

(followed by white light detection, matched with DAB color development kit, g1212 is recommended) or fluorescence (followed by fluorescence detection) according to the detection needs, and dilute it with PBS to prepare the secondary antibody working solution.

Assay Protocol / Procedures

Cell samples:

a. Cell preparation: prepare cell climbing pieces in advance to ensure normal cell state and good adhesion;

b. BrdU Incubation: BrdU containing cells are incubated in the incubator for a certain time in the absence of light. BrdU concentration and cell incubation time are different according to cell types. It is suggested to explore the best conditions in the pre experiment. The experimental conditions of the tested cells in the precautions are available for reference;

c. Cell fixation: the above cells incubated with BrdU were washed three times with PBS for 5 min each time. Add 1 ml of 4% paraformaldehyde to the well plate to cover the cells, and fix at room temperature for 20-30 min. Wash 3 times with PBS for 5 min each time;

d. Permeabilization: add membrane breaking solution to the well plate to cover the cells for 8-10 min, and wash with PBS for 3 times for 5 min each time;

e. Nuclear staining (optional):

In case of subsequent white light detection, the nuclei were stained with hematoxylin: add hematoxylin dye solution to the well plate for 5 min at room temperature, suck and discard hematoxylin dye solution, and wash with PBS for 3 times for 5 min each time;

In case of subsequent fluorescence detection, the nuclei were stained with DAPI: add DAPI dye solution to the well plate for 5 min at room temperature, suck and discard DAPI dye solution, and wash with PBS three times for 5 min each time;

f. Re fixation: add 4% paraformaldehyde dropwise to the well plate, incubate at room temperature for 10 min, and wash with PBS three times for 5 min each time;

g. Acidification: add 1 M HCl to the well plate to cover the cells, treat them at 37 ℃ for 10 min, and wash them with PBS three times for 5 min each time;

h. Post fixation: add 4% paraformaldehyde to the well plate again, incubate for 10 min at room temperature, and wash with PBS three times for 5 min each time;

i. Blocking: add blocking solution (3% BSA) to the well plate and incubate at room temperature for 30 min. Suck and discard the sealing liquid, and do not clean it;

j. Anti-BrdU antibody incubation: add anti BrdU primary antibody working solution to the well plate to cover the cells, and incubate overnight at 4 ℃. Suck and discard the anti BrdU primary antibody working solution, and wash it with PBS for 3 times for 5 min each time;

k. Secondary antibody incubation: add secondary antibody working solution to the well plate to cover the cells, and incubate at room temperature for 1H. Suck and discard the secondary antibody working solution and wash it with PBS for 3 times for 5 min each time. Note that if the fluorescent labeled secondary antibody is used, it should be protected from light during incubation and washing;

l. Seal and mirror inspection:

For HRP labeled secondary antibody, use DAB color reagent (recommended g1212) to develop the color of the cell sample, dehydrate and make it transparent. Gently pick up the climbing piece with pointed tweezers, buckle it upside down on the clean slide and seal it for microscope observation;

For the fluorescent labeled secondary antibody, add anti fluorescent quenching sealing agent (recommended g1401) onto the clean slide, gently lift the slide with pointed tweezers, and then buckle it

onto the slide for sealing, and observe with fluorescence microscope;

Tissue section samples:

a. Animal preparation: experimental animals shall be prepared in advance and BrdU labeling in vivo shall be carried out. Please refer to GC310002-100mg. After in vivo labeling, materials were taken, fixed and paraffin sections were prepared according to the experimental needs;

b. Tissue paraffin sections were dewaxed and rehydrated;

c. Repair: circle the tissue with group strokes, immerse the sections in the antigen repair solution, and heat them with microwave. Reference repair conditions: Granz microwave oven model P70D20TL-P4 (rated input power: 1180W; microwave output power: 700W), using 1 × Tris-EDTA antigen repair solution (pH 8.0, ready-to-use) (Item No. G1207), medium heat for 8min, cease heat for 8min, and turn to medium-low heat for 7min. the purpose of microwave repair is to keep the slices boiling for 15 minutes in the antigen repair solution. The purpose of microwave repair is to keep the slices boiling in the antigen repair solution for 15 minutes. Since the power of microwave ovens of different manufacturers is not the same, the time to reach boiling is not the same, and the specific repair conditions can be optimized according to the difference in power of microwave ovens. Natural cooling;

d. Nuclear staining (optional):

In case of subsequent white light detection, the nuclei were stained with hematoxylin: the sections were stained with hematoxylin at room temperature for 5 min, and washed with PBS for 3 times for 5 min each time;

In case of subsequent fluorescence detection, nuclei were stained with DAPI: DAPI dye solution was added dropwise to the tissue for 5 min at room temperature, DAPI dye solution was poured out, and sections were washed with PBS for 3 times for 5 min each time;

e. Re fixation: 4% paraformaldehyde was added dropwise to the tissue, incubated for 10 min at room temperature, and washed three times with PBS for 5 min each time;

f. Acidification: 1 M HCl was added dropwise to the sections to cover the tissues, treated at 37 ℃ for 10 min, and washed three times with PBS for 5 min each time;

g. Post fixation: add 4% paraformaldehyde to the sections again, cover the tissues, incubate them for 10 min at room temperature, and wash them with PBS for 3 times for 5 min each time;

h. Endogenous peroxidase quenching (optional): add 3% H2O2 to the sections and incubate at room temperature for 25-30min to remove endogenous peroxidase. Subsequently, the sections were washed three times with PBS for 5 min each time;

i. Blocking: add blocking solution (3% BSA) to the sections to cover the tissues, and incubate at room temperature for 30 min. Remove the sealing liquid and do not clean it;

j. Anti- BrdU antibody incubation: add anti BrdU primary antibody working solution to the sections to cover the tissues, and incubate at 4 ℃ overnight. The anti BrdU primary antibody working solution was removed and washed three times with PBS for 5 min each time;

k. Secondary antibody incubation: add secondary antibody working solution to the sections to cover the tissues, and incubate at room temperature for 1H. Remove the secondary antibody working solution and wash it three times with PBS for 5 min each time. Note that if the fluorescent labeled secondary antibody is used, it should be protected from light during incubation and washing;

l. Seal and mirror inspection:

If HRP labeled secondary antibody is used, DAB color reagent (recommended g1212) is used for color development of tissue sections, dehydration, transparency, and microscopic observation after sealing;

For fluorescent labeled secondary antibody, add anti fluorescence quenching sealing agent (recommended

g1401) dropwise and observe with fluorescence microscope after sealing.

Result Observation

When detected with HRP labeled secondary antibodies, the nuclei are dark blue and the proliferating cells are brown. If it is a fluorescent labeled secondary antibody, the nucleus shows blue fluorescence (ex = 358 nm, EM = 461 nm), and the proliferating cell is the fluorescence of the corresponding secondary antibody.

Note:

1. For cell climbing samples, the concentration and treatment time of BrdU were related to the cell type. Generally speaking, the concentration and time required to treat tumor cells are low. Cells with slow proliferation such as fibroblasts or epithelial cells need to increase the concentration and prolong the action time. The recommended concentration range is 20-100 μ M. The action time is 40 min-4 h, which can be adjusted according to the type of specific cells.

The following table shows the commonly used cell treatment concentration and time for testing, for reference only.

Cell	BrdU concentration（μM）	Incubation time（min）
A549	40	40
Hela	40	40
NRK-52e	40	40
SKOV3	80	120
H9C2	40	40

2. In order to ensure the best experimental effect, it is recommended to use other matching reagents produced by our company: hematoxylin dye solution (G1004); DAPI dye solution (G1012); DAB color development kit (G1212), anti fluorescence quenching sealing agent (G1401);

3. For your safety and health, please wear lab clothes and disposable gloves.

Servicebio® Cell Counting Kit-8

Cat. #：G4103

Product Information

Product Name	Cat. No	Spec.
Cell Counting Kit-8	G4103-1ML	1 mL
	G4103-5ML	5 mL

Introduction

Cell Counting Kit-8, abbreviated as CCK-8 kit or CCK8 kit, provides a rapid and highly sensitive method for measuring cell proliferation and cytotoxicity. The kit uses WST-8 compound that can be reduced by dehydrogenases in mitochondria to form water soluble formazan compound in orange color. The more the cells, the darker the color; the higher the cytotoxicity, the lighter the color. For the same cells, there is a linear relationship between color intensity and cell numbers. The WST-8 has no obvious toxicity to cells.The WST-8 in this kit has no obvious toxicity to cells, and can be used for the detection of cell proliferation induced by exogenous cytokines, as well as the detection of cytotoxicity induced by drugs and other cytotoxic reagents, or the detection of cell growth inhibition induced by some drugs. It is an upgraded version of the MTT detection method.

This kit is very convenient to use. It consists of a single tube of ready-to-use CCK-8 Solution containing WST-8. All steps are performed in the same well plate, without cell washing, collection and formazan dissolution. And this kit is compatible with phenol red and serum. It is suitable for both adherent cells and suspension cells.

Storage and Shipping Conditions

Ship with wet ice; store at 4℃ in the dark for up to 1 year, and -20℃ in the dark for up to 2 years.

Product Contents

Component	G4103-1ML	G4103-5ML
Cell Counting Kit-8	1 mL	5 mL
Manual		One copy

Assay Protocol / Procedures

1. Make a Standard Curve (Perform if measuring the exact number of cells is required)

A) Count the number of cells in the prepared cell suspension with a cytometer before seeding the cells.

B) Proportionally dilute the medium in equal proportions to form a cell concentration gradient. Generally, 3-5 cell concentration gradients are required, with 3-6 duplicate wells in each group.

C) Culture until the cells adhere to the wall, and then add CCK-8 reagent for a certain period of time to measure the OD value, and make a standard curve with the number of cells as the abscissa (X axis) and the OD value as the ordinate (Y axis). According to this standard curve, the number of cells in the unknown sample can be determined (the premise of using this standard curve is that the experimental conditions should be consistent, and it is convenient to determine the number of cells inoculated and the incubation time after adding CCK-8).

2. Cell Viability Assay

A) Seed cell suspension (100 μL/well) in a 96-well plate. Pre-incubate the plate in an incubator at 37°C with 5% CO2).

B) Add 10 μL of CCK-8 solution to each well (be careful not to create air bubbles in the wells, which will affect the OD value).

C) Incubate the culture plate in the incubator for 2 h.

D) Measure the absorbance at 450 nm with a microplate reader.

Note: If the OD value is not to be measured immediately,you can add 10 μL of 0.1M HCl or 1% SDS (W/V) solution to each well, and cover the culture plate and store it at room temperature in the dark. The absorbance did not change within 24 h.

3. Cell Proliferation-Toxicity Assay

A) Prepare 100 μL of cell suspension in a 96-well plate. Pre-incubate the plates for 24 hours in an incubator at 37 °C with 5% CO2).

B) Add 10 μL of different concentrations of the test compounds to the culture plate. Incubate for an appropriate period of time (eg: 6, 12, 24 or 48 hours).

C) Add 10 μL of CCK-8 solution to each well (be careful not to create air bubbles in the wells, which will affect the OD reading).

Note： If the test compounds is oxidative or reducing, the effect of the drug can be removed by replacing the medium with fresh medium (removing the medium, washing the cells twice with medium, then adding new medium) before adding CCK-8.

D) Incubate the plate in the incubator for 2 hours.

E) Measure the absorbance at 450 nm with a microplate reader.

F) If the OD value is not to be measured temporarily, if you plan to measure it later, you can add 10 μL of 0.1M HCl or 1% SDS (W/V) solution to each well, and cover the culture plate and store it at room temperature in the dark. The absorbance does not change within 24 hours.

4. Vitality Calculation

Cell viability (%) = [A (dosed) - A (blank)] / [A (0 dosed) - A (blank)] × 100

A (dosed): Absorbance of wells with cells, CCK-8 solution, and drug solution

A (blank): absorbance of wells with medium and CCK-8 solution without cells

A (0 dosing): Absorbance of wells with cells, CCK-8 solution and no drug solution

Cell viability: cell proliferative viability or cytotoxic viability

Note

1. This kit employs the reduction reaction catalyzed by dehydrogenase. Reducing agents or antioxidants that may interfere with the assay should be removed from samples to be tested.
2. It is recommended to perform pre-experiment to explore the number of inoculated cells and the incubation time after adding CCK-8 reagent.
3. Leukocytes may need to be cultured for a longer period of time.
4. When using standard 96-well plates, the minimum seeding volume of adherent cells is at least 1,000 cells / well (100 μL of medium). The sensitivity for detecting leukocytes is relatively low, it is recommend inoculate at least 2,500 cells / well (100 μL of medium). If you use a 24-well or 6-well plate, first calculate the corresponding inoculum amount of per well and add CCK-8 solution at 10% of the total volume of medium in each well.
5. If a 450 nm filter is not available, a filter with absorbance between 430-490 nm can be used, but 450 nm has the highest detection sensitivity.
6. The absorbance of phenol red in the medium can be eliminated by subtracting the absorbance of the background in the blank well during calculation, so it will not affect the detection.
7. Air bubbles in wells should be removed before the measurement of absorbance.
8. For your safty and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® Cell Counting Kit-8

Cat. #：G4103

Product Information

Product Name	Cat. No	Spec.
Cell Counting Kit-8	G4103-1ML	1 mL
	G4103-5ML	5 mL

Introduction

Cell Counting Kit-8, abbreviated as CCK-8 kit or CCK8 kit, provides a rapid and highly sensitive method for measuring cell proliferation and cytotoxicity. The kit uses WST-8 compound that can be reduced by dehydrogenases in mitochondria to form water soluble formazan compound in orange color. The more the cells, the darker the color; the higher the cytotoxicity, the lighter the color. For the same cells, there is a linear relationship between color intensity and cell numbers. The WST-8 has no obvious toxicity to cells.The WST-8 in this kit has no obvious toxicity to cells, and can be used for the detection of cell proliferation induced by exogenous cytokines, as well as the detection of cytotoxicity induced by drugs and other cytotoxic reagents, or the detection of cell growth inhibition induced by some drugs. It is an upgraded version of the MTT detection method.

This kit is very convenient to use. It consists of a single tube of ready-to-use CCK-8 Solution containing WST-8. All steps are performed in the same well plate, without cell washing, collection and formazan dissolution. And this kit is compatible with phenol red and serum. It is suitable for both adherent cells and suspension cells.

Storage and Shipping Conditions

Ship with wet ice; store at 4℃ in the dark for up to 1 year, and -20℃ in the dark for up to 2 years.

Product Contents

Component	G4103-1ML	G4103-5ML
Cell Counting Kit-8	1 mL	5 mL
Manual		One copy

Assay Protocol / Procedures

1. Make a Standard Curve (Perform if measuring the exact number of cells is required)

A) Count the number of cells in the prepared cell suspension with a cytometer before seeding the cells.

B) Proportionally dilute the medium in equal proportions to form a cell concentration gradient. Generally, 3-5 cell concentration gradients are required, with 3-6 duplicate wells in each group.

C) Culture until the cells adhere to the wall, and then add CCK-8 reagent for a certain period of time to measure the OD value, and make a standard curve with the number of cells as the abscissa (X axis) and the OD value as the ordinate (Y axis). According to this standard curve, the number of cells in the unknown sample can be determined (the premise of using this standard curve is that the experimental conditions should be consistent, and it is convenient to determine the number of cells inoculated and the incubation time after adding CCK-8).

2. Cell Viability Assay

A) Seed cell suspension (100 μL/well) in a 96-well plate. Pre-incubate the plate in an incubator at 37°C with 5% CO2).

B) Add 10 μL of CCK-8 solution to each well (be careful not to create air bubbles in the wells, which will affect the OD value).

C) Incubate the culture plate in the incubator for 2 h.

D) Measure the absorbance at 450 nm with a microplate reader.

Note: If the OD value is not to be measured immediately,you can add 10 μL of 0.1M HCl or 1% SDS (W/V) solution to each well, and cover the culture plate and store it at room temperature in the dark. The absorbance did not change within 24 h.

3. Cell Proliferation-Toxicity Assay

A) Prepare 100 μL of cell suspension in a 96-well plate. Pre-incubate the plates for 24 hours in an incubator at 37 °C with 5% CO2).

B) Add 10 μL of different concentrations of the test compounds to the culture plate. Incubate for an appropriate period of time (eg: 6, 12, 24 or 48 hours).

C) Add 10 μL of CCK-8 solution to each well (be careful not to create air bubbles in the wells, which will affect the OD reading).

Note： If the test compounds is oxidative or reducing, the effect of the drug can be removed by replacing the medium with fresh medium (removing the medium, washing the cells twice with medium, then adding new medium) before adding CCK-8.

D) Incubate the plate in the incubator for 2 hours.

E) Measure the absorbance at 450 nm with a microplate reader.

F) If the OD value is not to be measured temporarily, if you plan to measure it later, you can add 10 μL of 0.1M HCl or 1% SDS (W/V) solution to each well, and cover the culture plate and store it at room temperature in the dark. The absorbance does not change within 24 hours.

4. Vitality Calculation

Cell viability (%) = [A (dosed) - A (blank)] / [A (0 dosed) - A (blank)] × 100

A (dosed): Absorbance of wells with cells, CCK-8 solution, and drug solution

A (blank): absorbance of wells with medium and CCK-8 solution without cells

A (0 dosing): Absorbance of wells with cells, CCK-8 solution and no drug solution

Cell viability: cell proliferative viability or cytotoxic viability

Note

1. This kit employs the reduction reaction catalyzed by dehydrogenase. Reducing agents or antioxidants that may interfere with the assay should be removed from samples to be tested.
2. It is recommended to perform pre-experiment to explore the number of inoculated cells and the incubation time after adding CCK-8 reagent.
3. Leukocytes may need to be cultured for a longer period of time.
4. When using standard 96-well plates, the minimum seeding volume of adherent cells is at least 1,000 cells / well (100 μL of medium). The sensitivity for detecting leukocytes is relatively low, it is recommend inoculate at least 2,500 cells / well (100 μL of medium). If you use a 24-well or 6-well plate, first calculate the corresponding inoculum amount of per well and add CCK-8 solution at 10% of the total volume of medium in each well.
5. If a 450 nm filter is not available, a filter with absorbance between 430-490 nm can be used, but 450 nm has the highest detection sensitivity.
6. The absorbance of phenol red in the medium can be eliminated by subtracting the absorbance of the background in the blank well during calculation, so it will not affect the detection.
7. Air bubbles in wells should be removed before the measurement of absorbance.
8. For your safty and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® MTT Cell Proliferation and Cytotoxicity Assay Kit

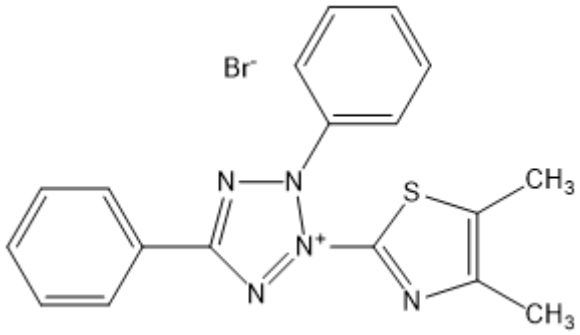
Cat.#：G4104

ProductInformation

ProductName	Cat.No.	Spec.
MTT Cell Viability Assay	G4104-100T	100T
	G4104-500T	500T

Description/Introduction

MTT is also called 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (Thiazolyl Blue Tetrazolium Bromide). Its molecular formula is $C_{18}H_{16}BrN_5S$, and the molecular weight is 414.32.



The MTT Cell Viability Assay utilizes the well-established and widely used MTT reagent to determine mammalian cell viability. The redox potential in active mammalian cells reduces MTT to a strongly pigmented formazan product. After solubilization, the absorbance of the formazan can be measured with a microplate absorbance reader. The MTT Cell Viability Assay is a complete, optimized kit that provides all the reagents necessary for the detection of mammalian cell viability.

The redox potential in viable mammalian cells causes the water soluble MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to convert to an insoluble formazan product. After solubilization of the formazan with the included SDS (sodium dodecyl sulfate) reagent, the concentration of the colorimetric probe is determined by an optical density measurement at 570 nm.

The MTT Cell Viability Assay provides a simple method for determination of mammalian cell viability using standard microplate absorbance readers. Simply prepare the MTT reagent, add it to the cells, solubilize the resulting formazan, and determine the optical density using a standard microplate reader.

Storage and Handling Conditions

Transport in wet ice; MTT solution, -20°C, protect from light; formazan dissolving agent, 4°C, protect from light; valid for 12 months.

Component

Component Number	Component	G4104-100T	G4104-500T
G4104-1	MTT Solution	1mL	5×1 mL
G4104-2	Formazan Dissolving Agent	10 mL	50 mL
Protocol		1	

Assay Protocol / Procedures

1. For cell proliferation assay, it is recommended to seed 100 μL of 2000 cells per well; for cytotoxicity experiments, it is recommended to seed 100 μL of 5000 cells per well (depending on cell size and proliferation rate). Culture and transfection or drug pretreatment according to the needs of the experiment;
2. Add 10 μL of MTT solution to each well and incubate for 4 h in an incubator;
3. a. Add 100 μL of formazan dissolving agent to the well plate,
b. tap the well plate to mix evenly
c. incubate in the incubator for 2-4 hours to dissolve the formazan (the incubation time depends on the number of cells)
4. Measure the absorbance of each well at 570 nm. If 570 nm filter is not available, the 560-600 nm filter can be used instead.

Note:

1. Use a 96-well plate for detection. If the cell culture time is long, it should be pay attention to the problem of evaporation. The circle around the 96-well plate is the easiest to evaporate. You can discard the circle and add PBS, water or culture medium instead. You can also ease the evaporation by placing the 96-well plate near the water source in the incubator.
2. In order to reduce the error caused by the experimental operation, it is recommended to set up duplicate wells to avoid the generation of air bubbles when adding samples or shaking and mixing.
3. The MTT solution is yellow. Before use, it should be placed at room temperature in the dark and water bath at 20-25°C for a while until it is completely melted before use. Prolonged exposure to light will cause failure, do not use if the solution is grey green.
4. If the formazan dissolving agent is precipitated, it can be dissolved in a water bath at room temperature or 37°C to promote dissolution, and then it can be used after dissolving and mixing.
5. For your health and safety, please wear a lab coat and disposable gloves when operating.

Servicebio® MTT Cell Proliferation and Cytotoxicity Assay Kit

Cat.#：G4104

Product information

product name	Identification of product	model
MTT Cell Proliferation and Cytotoxicity Assay Kit	G4104-100T	100T
	G4104-500T	500T

Descirption/Introduction

The MTT Cell Proliferation and Cytotoxicity Assay Kit provides a simple method for determining live cell numbers by absorbance on a microplate reader. MTT is a tetrazolium salt that is turned into a purple formazan product after reduction by mitochondrial enzymes that are only present in metabolically active live cells. The amount of formazan product generated is proportional to the number of living cells in the sample.

Determination of live cell numbers is often used to assess the rate of cell proliferation and cytotoxicity caused by drugs and cytotoxic agents. Among all non-radioactive viability assays, MTT assay is one of the most versatile and popular assays. At the end of the assay, the cells containing the formazan product are solubilized and then photometrically quantified at 570 nm.

In the traditional MTT assay, as the resulting purple compound formazan was water-insoluble, it was necessary to remove the upper layer of reaction liquid containing MTT substrate and then add DMSO to dissolve the purple compound formazan, which resulted in poor repeatability and reproducibility. This MTT Cell Proliferation and Cytotoxicity Assay Kit provides a special dissolver that can dissolve the water-insoluble formazan without removing the original MTT substrate reaction fluid.

Storage and Handling Conditions

Transport with wet ice, stored at -20℃. the formazan solvent can be stored at 4℃ protect from sunlight, valid for 12 months.

Product composition

Component Number	Component	G4104-100T	G4104-500T
G4104-1	MTT Reagent	1 mL	5×1 mL
G4104-2	Formazan Solvent	10 mL	50 mL
Manual		One copy	

Assay Protocol / Procedures

- Equilibrate all materials and prepared reagents to RT just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.
- Conditions such as age of the culture, number of passages, and growth media can affect the result and must be taken into consideration when analyzing the data. If your sample contains serum or phenol red, set up Sample Background Controls.

1. Grow cells at varying densities (1-5 x106 cells per mL) in a clear plate according to the desired protocol.

NOTE: Cells seeded at densities between 5,000-10,000 cells/well should reach optimal population densities within 48-72 hours. We recommend using appropriate incubation time depending on the individual cell type and cell concentration used.

2. If treating cells, dissolve compounds of interest in an appropriate solvent and treat cells with compound for desired time period.

NOTE: Prepare parallel well(s) as solvent control and use same volume of solvent as for the treated cells.

3. Add 10 μL of MTT Reagent into each well, incubate the plate at 37℃ for 4 hours.
4. After incubation, add 100 μL of Formazan Solvent into each well and mix thoroughly. Incubate at at 37℃ for 2-4 hours until the formazan was solubilized by microscope observation.
5. Read absorbance at OD=570 nm.
6. Data analysis:

(1) Cell proliferation assays:

-avarage the duplicate reading for each sample.

-subtract the culture medium background from your assay reading. This is the corrected absorbance.

-amount of absorbance is proportional to cell number.

(2) Cell cytotoxicity assays:

- average the duplicate reading for each sample.

- subtract the culture medium background from your assay readings. This is the corrected absorbance.

- calculate percentage cytotoxicity with the following equation, using corrected absorbance:

$$\%Cytotoxicity = \frac{100 \times \ (control - sample\ )}{control}$$

Note:

1. Equilibrate all the reagents to room temperature before use and open vial under sterile conditions.
2. If there is precipitation in Formazan Solvent, put in 37℃ water bath to promote dissolution and mixed before use.
3. For your safety and health, please wear lab coat and disposable gloves.

Ver. No.: V1.1-202206

Servicebio® EdU (5-ethynyl-2'-deoxyuridine)

Cat. #：G5059-100MG

Product Information

Product Name	Cat. No	Spec.
EdU (5-ethynyl-2'-deoxyuridine)	G5059-100MG	100 mg

Product Description/Introduction

EdU is a nucleoside analog of thymidine and is incorporated into DNA during active DNA synthesis. EdU can be used for animal in vivo injection. It has no obvious side effects on the organism and has good stability. When it is injected into animals, these small molecules can quickly diffuse into various organs and tissues in animals, and penetrate into cells, where cells proliferate. Instead of thymidine (T), it is incorporated into newly synthesized DNA, and then the target tissue can be prepared into paraffin or frozen tissue sections for detection; EdU is also suitable for the detection of cell proliferation in vitro, suitable for mice, rats and detection of EdU cell proliferation in various tissues and organs (except blood vessels) of other animal models. The acetylene group in the EdU molecule can undergo a "click" reaction with the fluorescently labeled azide probe under the catalysis of copper ions to form a stable, triazole ring, so the newly synthesized DNA can be labeled with the corresponding fluorescent probe.

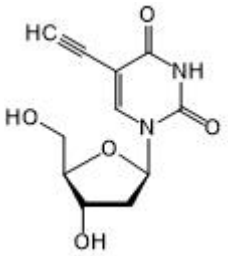
Compared with the radiolabeled nucleoside incorporation method, the EdU detection method has no limitations such as radioactive contamination; compared with BrdU-based assays, the EdU detection method does not require DNA denaturation, nor does it rely on antigen-antibody reaction, which allows for a simple, fast protocol producing more reproducible results. EdU cell proliferation assay has been widely used in in vitro cell or tissue cell proliferation assay.

Storage and Shipping Conditions

Ship with wet ice; store at -20°C and desiccate, valid for 12 months.

Product Contents

Name	5-Ethynyl-2'-deoxyuridine
Abbreviation	EdU
CAS No.	61135-33-9
MDL No.	MFCD01675687
Molecular Formula	$C_{11}H_{12}N_2O_5$
Molecular Weight	252.23
Form	Powder
Solubility	Soluble in water, DMSO, chloroform, etc.

Structural Formula	

Assay Protocol / Procedures

1. Centrifuge at low speed before use to ensure that the powder has fully settled to the bottom of the tube before opening the lid;
2. The recommended working concentration of EdU solution for cell proliferation is 1-50 μM, and DMSO is provided as a solvent to prepare a 10-50 mM stock solution, see Table 1 below;
3. The recommended EdU injection volume for animal modeling is 5 mg/kg (the ratio of EdU injection volume to animal body weight). The actual injection volume and marking time are determined according to the research content and animal conditions. Use PBS or physiological saline to prepare the final concentration of 0.5-1 mg/mL.

Table 1 Recommended Preparation Scheme of EdU Stock Solution

Final Concentration	1 mM	5 mM	10 mM	25 mM	50 mM
EdU	10 mg	10 mg	10 mg	10 mg	10 mg
DMSO	39.65 mL	7.93 mL	3.96 mL	1.59 mL	0.79 mL

Note

1. The product is harmful, please use with appropriate precautions.
2. The product is recommended to be used together with Click-iT EdU cell proliferation detection kit series (G1601, G1602, G1603, G1604).
3. For your safty and health,please wear safety glasses, gloves, or protective clothing.

Servicebio® 5-bromo-2'-deoxyuridine (BrdU)

Cat. # : GC310002

Product Information

Product Name	Cat. No.	Spec.
BrdU(5-Bromo-2'-deoxyUridine)	GC310002-100mg	100 mg

Product Description/Introduction

BrdU (5-bromo-2-deoxyuridine) is a thymidine analog that competes with thymidine for incorporation into DNA during the S phase of cell division. When cells in vigorous division are adulterated with BrdU, cells containing BrdU can be detected by using Anti-BrdU (mouse mAb) antibody in Anti-BrdU Detection Kit (G4102) in combination with the corresponding antibody ligase or fluorescein as an indication system. Double labeling by combining with other cellular markers can determine the types of proliferating cells, the rates of proliferation, etc., which is of great significance to the study of cellular dynamics. BrdU is incorporated into newly synthesized DNA in vivo injection or in cell cultures, with accurate positioning that reflects the level of DNA synthesis during the S phase. It is minimally affected by internal or external cellular environments, ensuring that labeled cells are not lost.

Main Parameters

CAS：59-14-3; **Molecular formula**：$C_9H_{11}BrN_2O_5$; **Molecular mass**：307.10; **Character**：white powder; **Purity**：≥99.0%.

Storage and Shipping Conditions

Ship with wet ice; Store away from light at -20 °C, valid for 36 months.

Handling Instruction

- Animal Experiment: BrdU powder was dissolved in physiological saline to make a concentration of 10 mg/mL, filtered and sterilized for later use. The dosage administered was generally 50 mg/kg, through intraperitoneal injection, given 4 times with a 2-hour interval. Animals were euthanized 24 hours after the final injection.
- Cell Experiment: The concentration and treatment time of BrdU usage for cells depend on the cell type. Generally, a lower concentration and shorter treatment time are required for tumor cells, while fibroblasts or epithelial cells with slower proliferation require higher concentrations and longer treatment times. It is recommended to use a concentration range of 20-100 μM and a treatment time of 40 min-4 h, which can be adjusted according to the specific cell type. The table below shows commonly used concentrations and times tested for reference purposes only.

Cell	BrdU Concentration (μM)	Incubation period (min)

A549	40	40
Hela	40	40
NRK-52e	40	40
SKOV3	80	120
H9C2	40	40

Note

1. Please avoid exposure to light when storing and preparing this product. Once prepared, it can be aliquoted and stored at -20℃.
2. We recommend using the G4102 BrdU assay kit for subsequent experiments.
3. For your safety and health, please wear laboratory attire and disposable gloves when handling this product.

Version: V1.0-202302

Servicebio® Hoechst 33258 Staining Solution (Ready-To-Use)

Cat No.: G1011-10ML

Product Information

Product Name	Cat.No.	Spec.
Hoechst 33258 Staining Solution (Ready-To-Use)	G1011-10ML	10 mL

Description

The molecular formula of Hoechst 33258 is C25H24N6O•3HCl, the molecular weight is 533.88, and the CAS number is 23491-45-4. Hoechst 33258 is a blue fluorescent dye that can penetrate cell membrane, and its fluorescence is significantly enhanced when combined with double-stranded DNA. The maximum excitation wavelength is 346 nm, and the maximum emission wavelength is 460 nm. Hoechst 33258 is commonly used for apoptosis detection, nuclear staining, or routine DNA staining. It can be used to stain the nuclei of fixed cells and tissues or non-fixed cells and tissues. After staining, it can be observed by fluorescence microscope or detected by flow cytometry. When observed by fluorescence microscope, it is excited by ultraviolet light, which is blue fluorescence.

This product is a ready-to-use solution, the concentration of which is optimized to meet the needs of all kinds of routine staining.

Storage and Handling Conditions

Wet ice transportation; Store at 2-8℃ away from light, valid for 6 months.

Component

Component	G1011
Hoechst 33258 Staining Solution (ready-to-use)	10 mL
Product Manual	1

Assay Protocol

1. For fixed cell or tissue sections:

a. For the fixed cell or tissue samples, remove the fixing solution after fixation, and wash with PBS or other appropriate buffer 2-3 times, 3-5 min each time (for the detection of suspended cells, please follow the routine operation method of suspended cells, and centrifugation and other steps shall be added in all experiments);

b. (Optional step) If the fixed cells or tissues need to be treated with immunofluorescence staining, the relevant treatment should be performed first, and then Hoechst 33258 staining should be performed after the treatment;

c. Directly take an appropriate amount of the product Hoechst 33258 dye solution (readyuse type) to cover the sample, and incubate at room temperature for about 5 min in the dark;

d. Remove the dye solution and wash it with PBS or other appropriate buffer 2-3 times, 3-5 min each time;

e. After sealing the plates or directly placed under a fluorescence microscope, the excitation wavelength was 350 nm, and the emission wavelength was 460 nm.

2. For living cells or cultured tissues:

a. Add an appropriate amount of Hoechst 33258 dye solution (ready-to-use type) to the cell culture to fully cover the sample to be stained; generally, 1 mL of staining solution should be added to a well of a 6-well plate, and 100 μL of staining solution

should be added to a well of a 96-well plate. The samples were incubated for 10-20 min in an incubator at 37℃.

b. Remove the dye solution and wash it with PBS or other appropriate buffer 2-3 times, 3-5 min each time;

c. Directly observed under a fluorescence microscope, the excitation wavelength was 350 nm, and the emission wavelength was 460 nm.

Note:

1. All fluorescent dyes have the problem of fluorescence quenching. It is recommended to take photos as soon as possible after dyeing. To slow down fluorescence quench, an anti-fluorescence quench sealer (G1401 recommended) can be used.

2. Wear a lab coat and disposable gloves during operation.

Servicebio® DAPI Staining Reagent (Ready to Use)

Cat. #：G1012

Product Information

Product Name	Cat. No	Spec.
DAPI Staining Reagent (Ready to Use)	G1012-10ML	10 mL
	G1012-100ML	100ML

Description

DAPI is 4', 6-diamamidine-2-phenylindole, is a common fluorescent dye, excitation wavelength of 340nm, emission wavelength of 488nm, and double-stranded DNA combined with the excitation wavelength of 364nm, emission wavelength of 454nm. DAPI can penetrate the intact cell membrane, and the fluorescence can be enhanced by more than 20 times after binding with double-stranded DNA. It is often used in the nuclear staining of living cells, fixed cells and tissue sections, and can detect cell apoptosis. The staining can be observed by fluorescence microscope or flow cytometry.

This product DAPI staining reagent (ready-to-use) concentration of 2 μg/mL, can be directly used for cell or tissue nuclear staining. The nucleus of the normal cell was bright blue with no fluorescence in the cytoplasm. When apoptosis occurred, the nuclei were densely stained or fragmented.

Storage and Handling Conditions

Wet ice transportation; Store at 2-8℃ away from light, valid for 12 months.

Component

Component	**G1012-10ML**	**G1012-100ML**
DAPI Staining Reagent (Ready to use)	10 mL	100 mL
Product Manual		

Assay Protocol

1. Cell crawl, cell smear or tissue section were rinsed with PBS (**recommended G4202**) for 3 times, 5 min each time.
2. Drop DAPI staining reagent (ready-to-use type) onto the section to completely cover the tissue, and incubate at room temperature for 8-10 min in the dark.
3. Rinse with PBS three times, 5 min each time.
4. Observe under fluorescence microscope or observe under fluorescence microscope after sealing.

Note:

1. All fluorescent dyes have the problem of fluorescence quenching. It is recommended to take photos as soon as possible after dyeing. To slow down fluorescence quenching, an anti-fluorescence quenching sealer (**G1401 is recommended**) can be used to seal the tablets.
2. Wear a lab coat and disposable gloves during operation.

Servicebio® PI Staining Solution

Cat. #：G1021-10ML

Product Information

Product Name	Cat. No	Spec.
PI Staining Solution	G1021-10ML	10 mL

Product Description/Introduction

PI, or propidium iodide, is an analog of ethidium bromide that binds strongly to DNA, and it releases red fluorescence upon embedding in double-stranded DNA to achieve staining of the DNA or nucleus. PI cannot penetrate intact cell membranes but can penetrate the broken cell membranes of late apoptotic cells and dead cells, and utilizing this feature, PI is usually used in combination with fluorescent probes such as Calcein-AM or FDA to stain and observe dead cells, or flow cytometry is used for relative quantitative detection of apoptosis and cell cycle. The PI-double-stranded DNA complex has a maximum excitation wavelength of 535 nm and a maximum emission wavelength of 615 nm.

The PI staining solution is a ready-to-use cell impermeable fluorescent solution with a concentration of 100 μg/mL. It can be directly used to stain the nuclei of necrotic cells or tissues, and the cell suspension can be used to detect cell cycle by flow cytometry after staining.

Storage and Shipping Conditions

Ship with wet ice ; store at 2-8℃ protecting from light, valid for 6 months.

Product Contents

Component	G1021-10ML
PI Staining Solution	10 mL
Manual	One copy

Assay Protocol / Procedures

I Flow cytometry assays for detection of cell cycle：

1. After digesting cells, wash cells with PBS, pellet cells by centrifugation at low-speed, and remove supernatant.
2. Slowly add 1-3 mL of 90% ethanol precooled at 20°C, resuspend cells. and incubate in ice bath for overnight.
3. Collect cells by centrifugation at 1,500 rpm for 5min, resuspended with PBS, and centrifuged again to remove the supernatant.
4. Add 250 μL PBS to resuspend the cells.
5. Add 2 μL of 1 mg/mL RNase A **(recommend G3405)** , and then incubate the mixture for 40 min in water bath at 37℃.
6. Add 50 μL PI Staining Solution and incubate for 20 min at room temperature, protected from light. (the length of time can be adjusted according to the staining results of experimental materials).

7. Detected by flow cytometry.

II Fluorescence Microscopy assays for identification of dead cells：

1. Remove the culture medium, and wash the cells twice with PBS.
2. Dilute the PI Staining Solution 1:20-1:10 in PBS to obtain a final concentration of 5-10 μg/mL PI staining working solution.
3. Add appropriate amount of PI staining working solution per well. Incubate for 5-10 minutes at room temperature, protected from light.
4. Remove the PI staining working solution. Add appropriate amount of PBS to each well and observed by fluorescence microscope.

Note: The nucleus of dead or late apoptotic cells show red observed by fluorescence microscope.

Note

1. All fluorescent dyes are quenched, and it is recommended to complete the detection on the same day after stained.
2. Prepare the working solution according to a 10-fold dilution, and add 0.2 mL of working solution dropwise per sample. This product can be used for approximately 500 stainings.
3. For your safety and health, please wear a lab coat and disposable gloves during operation.

Servicebio® Hoechst 33342 Staining Solution（1 mg/mL）

Cat. No.: G1127-1ML

Product Information

Product Name	Cat.No.	Spec.
Hoechst 33342 Staining Solution（1 mg/mL）	G1127-1ML	1 mL

Description

Hoechst 33342 (bisBenzimide H 33342, HOE 33342), the molecular formula is $C_{27}H_{28}N_6O \cdot 3HCl$, molecular weight is 561.93, CAS number is 23491-52-3. It is a blue fluorescent dye that can penetrate the cell membrane to stain DNA in the nucleus. This dye has weak fluorescence in the solution, but the fluorescence becomes brighter when combined with the small groove of the DNA in the AT-enriched area, so it is used for ordinary nuclear staining or conventional DNA staining.

This product is provided as a solution with a concentration of 1 mg/mL of Hoechst 33342. When used for staining, the usual working concentration is 0.5-10 μg/mL. Hoechst 33342 has a maximum excitation wavelength of 346 nm and a maximum emission wavelength of 460 nm; After being combined with double-stranded DNA, the maximum excitation wavelength is 350 nm and the maximum emission wavelength is 461 nm. It can be used to label the nucleus of cells or tissues under fixed or non-fixed conditions, and can be detected by fluorescence microscope or flow cytometer and other instruments.

Storage and Handling Conditions

Ship with wet ice； store at -20°C away from light, valid within 12 months。

Component

Component	G1127-1ML
Hoechst 33342 Staining Solution	1 mL
Manual	1 pc

Procedures

1. **The Hoechst 33342 staining solution (1 mg/mL)** was diluted to **0.5-10 μg/mL Hoechst 33342 staining working solution** with PBS or other suitable buffer;
2. **For fixed cell and tissue samples:**
   a. For fixed cell or tissue samples, remove the fixative after fixation and wash 2-3 times with PBS or other suitable buffer， 3-5 min each time (if detecting suspension cells, please follow the routine operation of suspension cells, and add steps such as centrifugation for all experiments);
   b. (optional) If the fixed cells or tissues need to be processed for immunofluorescence staining, etc., the relevant processing is prioritized, and the Hoechst 33342 staining is performed after the processing;

c. Take an appropriate amount of Hoechst 33342 staining working solution to stain the fixed cells or tissues, cover the sample and incubate at room temperature for about 5 minutes;

d. After removing the Hoechst 33342 staining working solution, wash with PBS or other suitable buffer 2-3 times for 3-5 min each time;

e. Sealing or observe it under a fluorescence microscope directly,Ex/Em: 350 nm/460 nm.

3. For living cells or cultured tissues:

a. Add an appropriate amount of Hoechst 33342 staining working solution to the sample to be stained. The sample must be fully covered. Incubate in a 37°C incubator for 10-20 min. Usually 1 mL of staining solution is added to one well of a six-well plate, 100 μL of staining solution should be added to one well of the 96-well plate;

b. Remove the staining solution,wash 2-3 times with PBS or other suitable buffer, 3-5 min each time;

c. Observe it under a fluorescence microscope directly,Ex/Em: 350 nm/460 nm.

Note

1. The incubation concentration and time of Hoechst 33342 staining working solution can be adjusted according to the actual situation.
2. Fluorescent dyes have quenching problems, try to complete the detection on the same day after dyeing; use anti-fluorescence quenching mounting tablets (**recommended Servicebio G1401**) to slow down fluorescence quenching.
3. For your health and safety, please wear lab coats and gloves during operation.

Servicebio® IF488-Phalloidin Stain

Cat. #：G1248

Product Information

Product Name	Cat. No	Spec.
IF488-Phalloidin Stain	G1248-100T	100 T

Introduction

Phalloidin, originally isolated as a bicyclic peptide from the poisonous mushroom Amanita phalloides, binds to actin filament F-actin with very high affinity and specificity, and does not bind monomeric actin (G-actin). Phalloidin have a similar affinity for small and large fibers, and essentially bind according to the stoichiometric ratio of one actin subunit to one phalloidin molecule in muscle and non-muscle cells of many different plant and animal species. Unlike actin antibodies, the affinity for motorized proteins changes markedly from one species or source to another. Nonspecific binding of phalloidin is almost negligible, and differences between stained and unstained regions are very pronounced. Phalloidin and their derivatives can stain F-actin at nanomolar concentrations, allowing very easy labeling, identification and quantitative study of F-actin distribution.

This product is a fluorescent dye IF488-labeled phalloidin, according to the use of 100 μL of working solution per well cell staining, this product can be used for 100 cell staining.

Storage and Handling Conditions

Transport with wet ice. Store at -20℃ away from light, valid for 12 months.

Components

Component	G1248-100T
IF488-Phalloidin Stain	100 T
Instruction Manual	1 pc

Pre-experiment preparation

1. Prepare your own **1×PBS buffer** (pH 7.2-7.4, recommended **G4202**), **BSA** (recommended **GC305010**), **fixative** (containing 4% paraformaldehyde, recommended **G1101**), **film-breaking solution** (0.1%-0.5% Triton X-100 dissolved in PBS, recommended **G1204**), **anti-fluorescence quenching sealer** (recommended **G1401**), **ready-to-use DAPI staining solution** (recommended **G1012**), etc.

2. When using this product for the first time, fully melt and centrifuge at low speed for 1 min to prevent loss of liquid tube wall. It is recommended to dispense the product according to the amount used in a single experiment to prevent loss of solvent by volatilization. Store at -20℃ away from light.

3. Before formal experiments, PBS containing 1% BSA was prepared, i.e., 1.0 g BSA was dissolved per 100 mL of PBS. 1 μL of IF488-Phalloidin Stain was then aspirated and mixed with 200 μL-500 uL of PBS containing 1% BSA to obtain 1× of the Phalloidin Staining Working Solution. The staining effect may be different for different types of cells, please refer to the literature for the optimal staining working concentration or perform the pre-test to find out. Use the Phalloidin Staining Solution on the same day as it is prepared and stored at room temperature and protected from light.

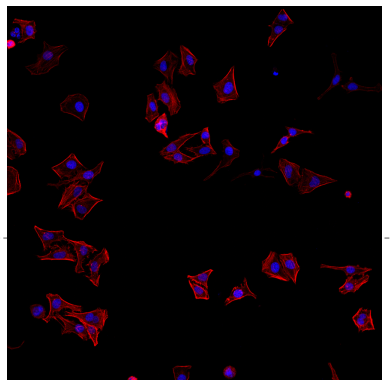
Usage

1. Cultured cells were crawled (at a density of at least half confluence), the culture medium was removed, and the cells were washed with 1× PBS buffer pre-warmed at 37°C to wash the cells 2 times;
2. Fixative containing 3.0-4.0% formaldehyde was added to cover the cells and fixed for 15-30 min at room temperature;
   Note: Methanol destroys actin, so the fixative must not contain methanol and avoid using formaldehyde solutions containing methanol;
3. Cells were washed with 1× PBS buffer 2-3 times for 10 min each at room temperature;
4. The cells were covered with PBS containing 0.1% Triton X-100 at room temperature and permeabilized for 5 min at room temperature;
5. Cells were washed with 1× PBS buffer 2-3 times for 10 min each at room temperature;
6. After the cell crawls were shaken dry slightly, draw a circle with a pap pen so that the cells were located in the center of the circle. Take 100 μL of the ready-made IF488-Phalloidin Staining Solution to cover the cells completely, and incubate at room temperature and away from light for 30-120 min. Note that normally, 4-37°C is suitable for staining. To avoid evaporation of the staining working solution leading to dry slices, the incubation process requires placing the cell crawls in a sealed container, such as a light-proof wet box. In addition, the addition of 1% BSA to the Phalloidin Staining Solution can effectively reduce the background;
7. Cells were washed with 1× PBS buffer 2-3 times for 5 min each time;
8. **(Optional)** Add drops of ready-to-use DAPI staining solution to the crawler slides to cover the cells, and perform nuclear staining for 3-5 min. wash the cells with 1× PBS buffer 2-3 times for 30-60 s each;
9. Cell crawls were slightly shaken dry and inverted on a slide with a drop of anti-fluorescence quenching sealer, and the for sealer was blotted off with a paper towel;
   [Optional step]: After completing step 7, the coverslip can also be inverted directly onto a slide titrated with an anti-fluorescence quenching sealer containing DAPI (**G1407 recommended**), and the excess sealer can then be aspirated off. Observe the results by fluorescence microscopy or laser confocal microscopy.
10. The results were observed by fluorescence microscopy or laser confocal microscopy with the IF488 (Ex/Em=493/517 nm) and DAPI (Ex/Em=364/454 nm) channels selected.

Note

1. One unit (T) of fluorescently labeled Phalloidin is defined as the amount of dye used to stain a slide of loaded cells.
2. Methanol can destroy actin protein, so fixation solution containing methanol should not be used in the early fixation of samples. The fixation time is recommended to be controlled between 15-30 min optimally.
3. Phalloidin is usually not permeable and is rarely used for live cell staining.
4. All fluorescent dyes are quenched, so it is recommended to complete the detection on the same day as possible after dyeing.
5. Phalloidin are toxic and should be handled with care. Wear a lab coat and disposable gloves during operation.

IF555-Phalloidin Stain



Cat.No. : G1249-100T

Brand : Servicebio

Spec.: 100 T （IF555）

Product Introduction

Product Information

Product Name	Cat. No	Spec.
IF555-Phalloidin Stain	G1249-100T	100 T

Introduction

Phalloidin, originally isolated as a bicyclic peptide from the poisonous mushroom Amanita phalloides, binds to actin filament F-actin with very high affinity and specificity, and does not bind monomeric actin (G-actin).

Phalloidin have a similar affinity for small and large fibers, and essentially bind according to the stoichiometric ratio of one actin subunit to one phalloidin molecule in muscle and non-muscle cells of many different plant and animal species.

Unlike actin antibodies, the affinity for motorized proteins changes markedly from one species or source to another. Nonspecific binding of phalloidin is almost negligible, and differences between stained and unstained regions are very pronounced.

Phalloidin and their derivatives can stain F-actin at nanomolar concentrations, allowing very easy labeling, identification and quantitative study of F-actin distribution.

This product is a fluorescent dye IF555-labeled phalloidin,
according to the use of 100 μL of working solution per well cell staining, this product can be used for 100 cell staining.

Storage and Handling Conditions

Transport with wet ice. Store at -20℃ away from light, valid for 12 months.

Components

Component	G1249-100T
IF555-Phalloidin Stain	100 T
Instruction Manual	1 pc

Servicebio® JC-1 Mitochondrial Membrane Potential Assay Kit

Cat. #：G1515

Product information

product name	Identification of product	model
JC-1 Mitochondrial Membrane Potential Assay Kit	G1515-100T	100T

Descirption/Introduction

JC-1 is a cationic carbonyl cyanine dye, which can pass through the cell membrane and aggregate towards mitochondria under the action of mitochondrial membrane potential. It is an ideal fluorescent probe widely used in detecting mitochondrial membrane potential. When the mitochondrial membrane potential is high, the dye aggregates in the matrix of mitochondria, showing red fluorescence(Ex=585 nm, Em=590 nm)； When the mitochondrial membrane potential is low, JC-1 is predominantly a monomer exist in cytoplasm, and showing green fluorescence(Ex=514 nm， Em=529 nm).Therefore, the change of mitochondrial membrane potential can be judged according to the transformation of fluorescence color and the change of proportion, and the decrease of mitochondrial membrane potential is also an important marker of early apoptosis.

JC-1 Mitochondrial Membrane Potential Assay Kit, is based on JC-1 fluorescent probe, which has improved easy precipitation problem and other shortcomings. This kit can be used to quickly and sensitively detect mitochondrial membrane potential changes in cell or purified mitochondria for determining the early apoptosis, and is also a common method used to detect early cell apoptosis. A total of 100 6-well plate samples can be tested in this kit.

In addition, this kit also provides CCCP (Carbonyl cyanide 3-chlorophenylhydrazone) reagent, a proton carrier (hydrogen ionophore) and decoupler of oxidative phosphorylation in mitochondria, which can promote changes in the permeability of mitochondria to hydrogen ions, resulting in the decrease or loss of mitochondrial membrane potential, which can be used as a positive control for inducing the decrease of mitochondrial membrane potential.

Storage and Handling Conditions

JC-1 dye（500×）should be stored at -20℃,desiccated and protected from light, and avoid repeated freezing and thawing;

JC-1 buffer and JC-1 diluent may be stored at 4℃. Valid for 12 months.

Component

Component Number	Component	G1515-100T
G1515-1	JC-1 dye（500 ×）	4 x 50 μL
G1515-2	JC-1 buffer (10 x)	50 mL
G1515-3	JC-1 diluent	100 mL
G1515-4	CCCP（100 mM）	50 μL
Manual		1 pc

Assay Protocol / Procedures

1. **prepare the working solution of JC-1 dye(2x) and JC-1 buffer(1x):**
   a. Take 2 μL of JC-1 solution (500×) and 900 μL of JC-1 dilution solution and mix well, then add 100 μL of JC-1 buffer (10×) and mix well by vortexing and shaking to prepare JC-1 working solution for spare use (take care to prepare this solution in order);

b. Take an appropriate amount of JC-1 buffer (10×) diluted with deionized water to formulate 1×JC-1 buffer and set aside (1×JC-1 buffer is used for subsequent steps, such as washing, if not otherwise specified).

2. prepare a positive control(optional):

a. Take an appropriate amount of CCCP (100 mM) and dilute 1000 times with cell culture medium to obtain 100 μM CCCP working solution;

b. Incubate the cells with CCCP working solution for about 30 minutes, and then follow the JC-1 staining procedure below to detect the mitochondrial membrane potential.

Note: For most of the cells, after treatment with the above treatments, compared with the normal group, JC-1 can be seen to exist mostly in the cytoplasm as monomers after induction by CCCP treatment, showing brighter green fluorescence and weaker red fluorescence; However, for some cells, the concentration and incubation time of CCCP may be different, please refer to reference or do experiment to find the optimal conditions.

3. Cell stain and analyze

a. Take 1-6×10^5 cells, centrifuge at 1000 g for 3-5 min to remove the original medium, add JC-1 buffer and wash once, then centrifuge to remove JC-1 buffer and resuspend with 500 μL of cell culture medium (serum and phenol red can be included).;

b. Then add 500 μL of JC-1 working solution and incubate in a CO_2 incubator for 15-30 min (generally 20 min is sufficient, or the incubation time can be adjusted according to the situation), protected from light;

c. At the end of the incubation, the cells were still treated as conventional suspension cells, and after centrifugation to remove the JC-1 working solution, the cells were washed twice with JC-1 buffer ;

d. After resuspension in appropriate amount of JC-1 buffer (1×) or cell culture solution (phenol red free is recommended), it was observed by fluorescence microscope or laser confocal microscope, or analyzed by flow cytometry and other instruments..

4. Adherent cell staining operation (6-well plate as an example):

For adherent cells, if they need to be detected by flow cytometry, the cells can be collected first, resuspended and then referred to the assay for suspension cells.

a. After removing the original cell culture medium containing drugs or other treatments, add 1 mL of JC-1 buffer and wash 1-2 times;

b. Add 1 mL of cell culture medium (may contain serum and phenol red);

c. Add 1 mL of JC-1 working solution, shake gently to mix well, and incubate for 15-30 min in a CO_2 incubator protected from light;

d. At the end of incubation, the supernatant was aspirated, and with JC-1 buffer, washed twice;

e. Add 2 mL of JC-1 buffer (1×) or cell culture solution (phenol red free recommended);

f. Observed under a fluorescence microscope or laser confocal microscope or analyzed by an instrument such as a flow cytometer.

5. For the extraction of purified mitochondria

a. 900 μL of JC-1 working solution was supplemented with 100 μL of total protein amounting to 10-100 μg of purified mitochondria;

b. After mixing, it was scanned and detected using an instrument such as a fluorescence spectrophotometer (with an excitation wavelength of 485 nm and an emission wavelength of 590 nm) or an enzyme labeling instrument (when the excitation wavelength could not be set to

485 nm, the excitation wavelength could be set within the range of 475-520 nm), or it could be observed by fluorescence or laser confocal microscopy, which could be detected with reference to the wavelength setting in step 6.

6. Fluorescence detection and analysis

a. The wavelength parameters for detecting JC-1 monomer are Ex=490 nm, Em=525 nm; the wavelength parameters for JC-1 polymer are Ex=525 nm, Em=590 nm; note that it is not necessary to set the excitation and emission at the maximum excitation and emission wavelengths in the determination of fluorescence here, and that the parameter settings can be adjusted according to the situation around this parameter;

b. When taking pictures with fluorescence microscope, it is recommended to use the normal group as a standard to determine the exposure time for red and green fluorescence, so that the red fluorescence effect of the normal group is clear and the green fluorescence effect is darker, and to take pictures of the fluorescence of the processing group with the exposure time for red and green fluorescence of the normal group, respectively;

c. Judgment of the results: the cell state is normal, mainly showing red fluorescence, indicating that its mitochondrial membrane potential is normal; if there is a significant increase in green fluorescence, indicating that the mitochondrial membrane potential has declined, which may be in the early stage of apoptosis.

Note

1. **In order to fully dissolve JC-1, follow the sequence of operation for JC-1 working solution preparation.**
2. It is recommended to test the samples within 30 min after JC-1 incubation.
3. JC-1 solution, JC-1 buffer (10×) can be dissolved in an appropriate warm bath if precipitation is produced.
4. **For JC-1 final assay, it is recommended to use phenol red free cell culture solution** to avoid background color interference, and phenol red at the incubation stage of JC-1 staining has no effect on the results.
5. If less JC-1 solution is used at a time, which may involve multiple repeated freezing and thawing, please dispense as appropriate and try to avoid multiple repeated freezing and thawing of JC-1 solution.
6. Please wear a lab coat and disposable gloves in operation.

Servicebio® DiO Cell Membrane Green Fluorescence Staining Kit

Cat. #：G1704

Product Information

Product Name	Cat. No	Spec.
DiO Cell Membrane Green Fluorescence Staining Kit	G1704	100-1000 T

Product Description/Introduction

DiO, also known as DiOC18(3), 3-octadecyl-2-[3-(3-octadecyl-2(3H)-benzoxazol-2-ylidene)-1-propen-1-yl]benzoxazole perchlorate, with a molecular weight of 881.72, is a class of lipophilic, long-chain dialkyldicarbocyanine dyes, fluorescent dyes, commonly used in the labeling of cell membranes and other fat-soluble biological structures. After entering the cell membrane, DiO can diffuse laterally and gradually stain the whole cell membrane.DiO fluorescence is very weak before entering the cell membrane, and the fluorescence intensity will be greatly enhanced when it binds to the cell, and it can emit green fluorescence after excitation and can be detected by standard FITC filters. The maximum excitation wavelength of DiO is 484nm, and the maximum emission wavelength is 501nm. According to the characteristics of DiO, it can stain living cells as well as fixed cells. In addition. DiO probes generally do not affect the viability of cells, so forward or reverse labeled cells or some substances (exosomes) can be used as tracer detection.

The DiO Cell Membrane Green Fluorescence Staining Kit contains DiO fluorescence probe and optimized staining buffer, which can make cell membrane staining faster, fluorescence more bright and stable.

Storage and Shipping Conditions

Ship with dry ice; store at -20°C in the dark, valid for 6 months.

Product Contents

Component Number	Component	G1704
G1704-1	DiO cell membrane green fluorescent probe	400 μL
G1704-2	Staining buffer	100 mL
Manual		One copy

Assay Protocol / Procedures

1. Preparation of DiO staining working solution:

1.1. Mix and dilute DiO Cell Membrane Green Fluorescent Probe with Staining Buffer at a ratio of 1:250 to prepare DiO Staining Working Solution (ready to use); note that the DiO probe dilution ratio can be adjusted at 1:250-1:500 according to the specific situation in order to obtain the best staining effect.

2. Staining of live suspension cells

2.1. Centrifuge the suspended cells at 500-1,000×g at room temperature for 3-5 min, remove the cell supernatant;

2.2. Add appropriate amount of Dio staining working solution to resuspend cells to a final cell density at 1-2×10^6 cells/mL.

2.3. Incubate at 37℃ in the dark for 5-20 min. The optimal incubation time varies for different cells. Start with 5 min and optimize the incubation time based on the final staining result.

2.4. At the end of incubation, centrifuge at 500-1000 g for 3-5 min at room temperature and aspirate the DiO staining working solution;

2.5. Cells were resuspended with pre-warmed PBS or cell culture medium at 37°C and centrifuged at 500-1000 g for 3-5 min to remove the supernatant;

2.6. Repeat step 2.5.

2.7. Detected by flow cytometry directly or by fluorescence microscopy after transfering cells to a multi-well plate, cell culture dish or cell climbing slide. Dio has an excitation maximum at 484 nm and an emission maximum at 501 nm.

3. Staining of live adherent live cells (6-well plate as an example):

3.1. Seed adherent cells in 6-well plate at a certain density.

3.2. Remove the culture medium and wash cells twice with PBS (Recommend G4202). Add 1 mL of Dio staining working solution (other size well plates, adjusted as appropriate to ensure that the dye covers the cells);

3.3. Incubate at 37℃ in the dark for 5-20min. The optimal incubation time varies for different cells. Start with 5 min and optimize the incubation time based on the final staining result.

3.4. Aspirate the cell membrane staining working solution and wash cells 1-2 times with preheated PBS or cell medium.

3.5. Add pre-warmed cell culture medium or cell medium at 37℃ and detect cells by fluorescence microscopy. Dio has an excitation maximum at 484 nm and an emission maximum at 501 nm.

4. Staining of fixed adherent cells:

4.1. Sample preprocessing:

For cells: Remove the cell medium and wash 1-2 times with PBS. Add 4% paraformaldehyde fix solution (Recommend G1101) for 10 min at room temperature. Remove fix solution and wash 2-3 times with PBS;

4.2. Permeabilization: Add 0.1-0.5% Triton-100 (prepared with PBS) and permeated for 10 min at room temperature. Remove permeabilization solution and wash 2-3 times with PBS.

4.3. (Optional, immunofluorescent labeling) Incubate with antibodies according to immunostaining protocol or incubate with other dyes.

Note: Blocking solution, antibody diluent, and wash solution for immunostaining should not contain detergents.

4.4. Add an appropriate amount of DiO staining working solution to cover the cells, incubate at 37°C away from light for 5-20 min, and aspirate the DiO staining working solution. It is recommended that the staining time be adjusted to the specific cell sample to obtain optimal staining results;

4.5. Cells were washed 2-3 times with PBS and then placed under a fluorescence microscope for observation (cells need to be covered with appropriate amount of PBS).DiO has a maximum excitation wavelength of 484nm and a maximum emission wavelength of 501nm.

5. Fluorescent labeling of exosomes:

5.1. Resuspended exosome precipitation with an appropriate amount of DiO staining working solution.

5.2. Incubate at 37℃ for 30 min in the dark.

5.3. (optional) Dilute the sample with 10-fold volume PBS.

5.4. Extracted exosomes again according to the previous extraction protocol to remove excess dyes.

5.5. Collect exosomes precipitation and resuspended in PBS to obtain DIO-labeled exosomes. It can be used for subsequent experiments, such as cellular uptake.

1. All fluorescent dyes have quenching problems, please protect from light during the operation to slow down fluorescence quenching.
2. Due to the probe is lipophilic, please avoid using reagents containing glycerin or other organic matter.
3. If fixation is required, we recommend to fix in 4% paraformaldehyde. Other inappropriate fix solutions will lead to high fluorescence background.
4. The optimum dilution ratio and incubation time of the probe should be adjusted according to the actual situation due to the different sensitivity between cells and experimental requirement.
5. For your safty and health, please wear safety glasses, gloves, or protective clothing.

Ver. No.: V2.0-202308

Servicebio® Dil Cell Membrane Red Fluorescence Staining Kit

Cat. #：G1705

Product Information

Product Name	Cat. No	Spec.
Dil Cell Membrane Red Fluorescence Staining Kit	G1705	100-1000 T

Product Description/Introduction

Dil, also known as DilC18(3), 1,1`-Dioctadecyl-3,3,3`,3`-tetramethylindocarbocyanine perchlorate, with a molecular weight of 933.87, is a class of lipophilic, long-chain dialkyldicarbocyanine dyes, fluorescent dyes, commonly used in the labeling of cell membranes and other fat-soluble biological structures. After entering the cell membrane, Dil can diffuse laterally and gradually stain the whole cell membrane.Dil fluorescence is very weak before entering the cell membrane, and the fluorescence intensity will be greatly enhanced when it binds to the cell, and it can emit orange-red fluorescence after excitation and can be detected by standard TRITC filters. The maximum excitation wavelength of Dil is 550 nm, and the maximum emission wavelength is 564 nm. According to the characteristics of Dil, it can stain living cells as well as fixed cells. In addition. Dil probes generally do not affect the viability of cells, so forward or reverse labeled cells or some substances (exosomes) can be used as tracer detection.

The Dil Cell Membrane Red Fluorescence Staining Kit contains Dil fluorescence probe and optimized staining buffer, which can make cell membrane staining faster, fluorescence more bright and stable.

Storage and Shipping Conditions

Ship with dry ice; store at -20°C in the dark, valid for 6 months.

Product Contents

Component Number	Component	G1704
G1705-1	Dil Cell Membrane Red Fluorescent Probe	100μL
G1705-2	Staining Buffer	100mL
Manual		One copy

Assay Protocol / Procedures

1. Preparation of Dil staining working solution:

1.1. Mix and dilute Dil Cell Membrane Red Fluorescent Probe with Staining Buffer at a ratio of 1:250 to prepare Dil Staining Working Solution (ready to use); note that the Dil probe dilution ratio can be adjusted at 1:250-1:500 according to the specific situation in order to obtain the best staining effect.

2. Staining of live suspension cells

2.1. Centrifuge the suspended cells at 500-1,000×g at room temperature for 3-5 min, remove the cell supernatant;

2.2. Add appropriate amount of Dil staining working solution to resuspend cells to a final cell density at 1-2×10^6 cells/mL.

2.3. Incubate at 37℃ in the dark for 5-20 min. The optimal incubation time varies for different cells. Start with 5 min and optimize the incubation time based on the final staining result.

2.4. At the end of incubation, centrifuge at 500-1000 g for 3-5 min at room temperature and aspirate

the DiI staining working solution;

2.5. Cells were resuspended with pre-warmed PBS or cell culture medium at 37°C and centrifuged at 500-1000 g for 3-5 min to remove the supernatant;

2.6. Repeat step 2.5.

2.7. Detected by flow cytometry directly or by fluorescence microscopy after transfering cells to a multi-well plate, cell culture dish or cell climbing slide. DiI has an excitation maximum at 550 nm and an emission maximum at 564 nm.

3. Staining of live adherent live cells (6-well plate as an example):

3.1. Seed adherent cells in 6-well plate at a certain density.

3.2. Remove the culture medium and wash cells twice with PBS (Recommend G4202). Add 1 mL of DiI staining working solution (other size well plates, adjusted as appropriate to ensure that the dye covers the cells);

3.3. Incubate at 37℃ in the dark for 5-20min. The optimal incubation time varies for different cells. Start with 5 min and optimize the incubation time based on the final staining result.

3.4. Aspirate the cell membrane staining working solution and wash cells 1-2 times with preheated PBS or cell medium.

3.5. Add pre-warmed cell culture medium or cell medium at 37℃ and detect cells by fluorescence microscopy. DiI has an excitation maximum at 550 nm and an emission maximum at 564 nm.

4. Staining of fixed adherent cells:

4.1. Sample preprocessing:

For cells: Remove the cell medium and wash 1-2 times with PBS. Add 4% paraformaldehyde fix solution (Recommend G1101) for 10 min at room temperature. Remove fix solution and wash 2-3 times with PBS;

4.2. Permeabilization: Add 0.1-0.5% Triton-100 (prepared with PBS) and permeated for 10 min at room temperature. Remove permeabilization solution and wash 2-3 times with PBS.

4.3. (Optional, immunofluorescent labeling) Incubate with antibodies according to immunostaining protocol or incubate with other dyes.

Note: Blocking solution, antibody diluent, and wash solution for immunostaining should not contain detergents.

4.4. Add an appropriate amount of DiI staining working solution to cover the cells, incubate at 37°C away from light for 5-20 min, and aspirate the DiI staining working solution. It is recommended that the staining time be adjusted to the specific cell sample to obtain optimal staining results;

4.5. Cells were washed 2-3 times with PBS and then placed under a fluorescence microscope for observation (cells need to be covered with appropriate amount of PBS).DiI has a maximum excitation wavelength of 550 nm and a maximum emission wavelength of 564 nm.

5. Fluorescent labeling of exosomes:

5.1. Resuspended exosome precipitation with an appropriate amount of DiI staining working solution.

5.2. Incubate at 37℃ for 30 min in the dark.

5.3. (optional) Dilute the sample with 10-fold volume PBS.

5.4. Extracted exosomes again according to the previous extraction protocol to remove excess dyes.

5.5. Collect exosomes precipitation and resuspended in PBS to obtain DiI-labeled exosomes. It can be used for subsequent experiments, such as cellular uptake.

Note

1. All fluorescent dyes have quenching problems, please protect from light during the operation to slow down fluorescence quenching.

2. Due to the probe is lipophilic, please avoid using reagents containing glycerin or other organic matter.
3. If fixation is required, we recommend to fix in 4% paraformaldehyde. Other inappropriate fix solutions will lead to high fluorescence background.
4. The optimum dilution ratio and incubation time of the probe should be adjusted according to the actual situation due to the different sensitivity between cells and experimental requirement.
5. For your safty and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® ER-Tracker Green

Cat. No.: G1720-20UL

Product Information

Product Name	Cat. No.	Spec.
ER-Tracker Green	G1720-20UL	20 μL

Product Description/Introduction

ER-Tracker green (Endoplasmic Reticulum Green Fluorescent Probe) is a combination of the green fluorescent dye BODIPY FL and Glibenclamide. The glibenclamide group in its chemical structure, originally a type II diabetes treatment drug, binds to the sulfonylurea receptor of ATP-sensitive potassium channels on the endoplasmic reticulum, and thus can be selectively localized to the endoplasmic reticulum and used as an endoplasmic reticulum-specific fluorescent probe for labeling the endoplasmic reticulum of cells.ER tracker green has good specificity, is highly selective for the endoplasmic reticulum of living cells, rarely stains mitochondria, and has low cytotoxicity. This probe can partially retain the staining characteristics of living cells after staining and fixation.

Storage and Shipping Conditions

Ship with dry ice; Store at -20℃ away from light for 12 months.

Product Content

Component	G1720-20UL
ER-Tracker Green	20 μL
Manual	1 pc

Assay Protocol / Procedures

1. **Assay working solution preparation:**
   - 1.1. This product is a 1 mM storage solution that is warmed back to room temperature prior to experimentation and centrifuged briefly at low speed to ensure that the reagents sink to the bottom of the tube;
   - 1.2. It is diluted to 0.25-1 μM working solution for endoplasmic reticulum detection by buffer (Hanks, PBS, etc., Hanks is recommended) or basal medium (without serum);
2. **Cell staining** (the following protocol is for adherent cells; centrifugation is required for suspension cell exchange)
   - 2.1. Normal or treated cells are washed 1-2 times with buffer for 3-5 min each time;
   - 2.2. Add pre-warmed endoplasmic reticulum assay working solution, and incubate in a cell culture incubator at 37°C for 20 min (the incubation time can be adjusted to some extent due to different cell types and states);
   - 2.3. Remove the endoplasmic reticulum assay working solution and wash with buffer 2-3 times for 3-5 min each;
   - 2.4. Green fluorescence (Ex=504 nm, Em=511 nm) is observed under a fluorescence microscope after blocking the film directly or with an Anti-fade Mounting Medium (recommended G1401);
3. **Cell fixation (optional):**

   Note: After staining, this product can be fixed, but the fluorescent signal will be attenuated after fixation; it cannot be perforated for processing, and perforation will lead to the disappearance of

fluorescent labeling.

3.1. The stained and washed treated cells are added to the fixative (recommended G1101) and fixed for 5 min at room temperature;

3.2. Remove the fixative and wash with buffer 2-3 times for 3-5 min each time;

3.3. After washing, the cells can be subsequently blocked, microscopically examined or further stained according to experimental needs.

Note

1. Due to the different types and states of the cells, the working concentration of the dye, and the incubation time, can be adjusted appropriately.
2. The probe could not be perforated for treatment after staining, and cell fluorescence disappeared almost completely after perforation.
3. The pharmacologic properties of glibenclamide may affect certain functions of the endoplasmic reticulum; variable expression of sulfonylurea receptors in some specialized cells may also result in non-endoplasmic reticulum-specific staining.
4. When staining and washing live cells, the assay working solution and washing buffer need to be pre-warmed at 37°C. Staining and washing are accomplished by incubation in an incubator so as not to affect the morphology of the cells by temperature difference stimulation.
5. In order to prevent fluorescence quenching, the entire staining process needs to be operated away from light.
6. Probe master mixes should be stored in separate containers to avoid repeated freezing and thawing.To avoid waste, the staining solution is prepared as needed, ready to use..
7. For your health and safety, please wear lab coat and gloves during operation.

Servicebio® ER-Tracker Red

Cat. No.: G1721-20UL

Product Information

Product Name	Cat. No.	Spec.
ER-Tracker Red	G1721-20UL	20 μL

Product Description/Introduction

ER-Tracker Red (Endoplasmic Reticulum Red Fluorescent Probe) is a combination of the red fluorescent dye BODIPY FL and Glibenclamide. The glibenclamide group in its chemical structure, originally a type II diabetes treatment drug, binds to the sulfonylurea receptor of ATP-sensitive potassium channels on the endoplasmic reticulum, and thus can be selectively localized to the endoplasmic reticulum and used as an endoplasmic reticulum-specific fluorescent probe for labeling the endoplasmic reticulum of cells.ER tracker red has good specificity, is highly selective for the endoplasmic reticulum of living cells, rarely stains mitochondria, and has low cytotoxicity. This probe can partially retain the staining characteristics of living cells after staining and fixation.

Storage and Shipping Conditions

Ship with dry ice; Store at -20℃ away from light for 12 months.

Product Content

Component	G1721-20UL
ER-Tracker Red	20 μL
Manual	1 pc

Assay Protocol / Procedures

1. **Assay working solution preparation:**
   - 1.1. This product is a 1 mM storage solution that is warmed back to room temperature prior to experimentation and centrifuged briefly at low speed to ensure that the reagents sink to the bottom of the tube;
   - 1.2. It is diluted to 0.25-1 μM working solution for endoplasmic reticulum detection by buffer (Hanks, PBS, etc., Hanks is recommended) or basal medium (without serum);
2. **Cell staining** (the following protocol is for adherent cells; centrifugation is required for suspension cell exchange)
   - 2.1. Normal or treated cells are washed 1-2 times with buffer for 3-5 min each time;
   - 2.2. Add pre-warmed endoplasmic reticulum assay working solution, and incubate in a cell culture incubator at 37°C for 20 min (the incubation time can be adjusted to some extent due to different cell types and states);
   - 2.3. Remove the endoplasmic reticulum assay working solution and wash with buffer 2-3 times for 3-5 min each;
   - 2.4. Red fluorescence (Ex=587 nm, Em=615 nm) is observed under a fluorescence microscope after blocking the film directly or with an anti-fluorescence bursting agent (recommended G1401);
3. **Cell fixation (optional):**

   Note: After staining, this product can be fixed, but the fluorescent signal will be attenuated after fixation; it cannot be perforated for processing, and perforation will lead to the disappearance of

fluorescent labeling.

3.1. The stained and washed treated cells are added to the fixative (recommended G1101) and fixed for 5 min at room temperature;

3.2. Remove the fixative and wash with buffer 2-3 times for 3-5 min each time;

3.3. After washing, the cells can be subsequently blocked, microscopically examined or further stained according to experimental needs.

Note

1. Due to the different types and states of the cells, the working concentration of the dye, and the incubation time, can be adjusted appropriately.
2. The probe could not be perforated for treatment after staining, and cell fluorescence disappeared almost completely after perforation.
3. The pharmacologic properties of glibenclamide may affect certain functions of the endoplasmic reticulum; variable expression of sulfonylurea receptors in some specialized cells may also result in non-endoplasmic reticulum-specific staining.
4. When staining and washing live cells, the assay working solution and washing buffer need to be pre-warmed at 37°C. Staining and washing are accomplished by incubation in an incubator so as not to affect the morphology of the cells by temperature difference stimulation.
5. In order to prevent fluorescence quenching, the entire staining process needs to be operated away from light.
6. Probe master mixes should be stored in separate containers to avoid repeated freezing and thawing.To avoid waste, the staining solution is prepared as needed.
7. For your health and safety, please wear lab coat and gloves during operation.

Servicebio® Lyso-Tracker Green

Cat. No.: G1722-50UL

Product Information

Product Name	Cat. No.	Spec.
Lyso-Tracker Green	G1722-50UL	50 μL

Product Description/Introduction

Lyso-Tracker Green (DND-26), a lysosomal green fluorescent probe, can be used to specifically stain lysosomes in living cells. The probe is composed of a fluorescent group and connected weak bases, which can freely penetrate into cells and aggregate towards lysosomes with a weakly acidic internal environment, thus realizing the labeling of lysosomes.

Storage and Shipping Conditions

Ship with dry ice; Store at -20℃ away from light for 12 months.

Product Content

Component	G1722-50UL
Lyso-Tracker Green	50 μL
Manual	1 pc

Assay Protocol / Procedures

1. **Assay working solution preparation:**
   - 1.1. This product is a 1 mM storage solution that is warmed back to room temperature prior to experimentation and centrifuged briefly at low speed to ensure that the reagents sink to the bottom of the tube;
   - 1.2. It is diluted by buffer (Hanks, PBS, etc., Hanks is recommended) or basal medium (without serum), into a lysosomal assay working solution of 50-100 nM (Lyso-Tracker Green Fluorescent Probe Working Concentration, can be adjusted to some extent depending on the situation, but in order to avoid too high a concentration of probe, which will lead to a decrease in the specificity of the probe, resulting in false positives and so on, it is recommended that a lower concentration be used);
2. **Cell staining** (the following protocol is for adherent cells; centrifugation is required for suspension cell exchange)
   - 2.1. Normal or treated cells are washed 1-2 times with buffer for 3-5 min each time;
   - 2.2. Add pre-warmed lysosomal assay working solution and incubate in a cell culture incubator at 37℃ for 30-60 min (it is recommended to observe the fluorescence effect under a fluorescence microscope first, if the effect is not obvious, the incubation time can be extended appropriately);
   - 2.3. Remove the incubation solution and wash with buffer solution 2-3 times for 3-5 min each time;
3. **Microscopic examination：** Ready for observation under a fluorescence microscope, FITC filter was selected with excitation wavelength of 504 nm and emission wavelength of 511 nm..

Note

1. Due to the different types and states of the cells, the working concentration of the dye, and the incubation time, can be adjusted appropriately; The study of the internalization kinetics of the probes

has shown that it takes only a few seconds for the probes to enter the living cells. It is important to note that the probe causes an "alkalinization effect" in the lysosome, and therefore prolonged incubation may cause an increase in lysosomal pH.

2. The dye needs to perform lysosomal localization in the state of living cells, and it is not suitable for subsequent cell fixation operations.
3. When staining and washing live cells, the assay working solution and washing buffer need to be pre-warmed at 37°C. Staining and washing are accomplished by incubation in an incubator so as not to affect the morphology of the cells by temperature difference stimulation.
4. The fluorescence of this probe is easily quenched, so it should be photographed as soon as possible after staining, and the whole staining process needs to be operated away from light.
5. Probe master mixes should be stored in separate containers to avoid repeated freezing and thawing.To avoid waste, the staining solution is prepared as needed, ready to use.
6. For your health and safety, please wear lab coat and gloves during operation.

Servicebio® Mito Tracker Red CMXRos

Cat. No.: G1723-50UG

Product Information

Product Name	Cat. No.	Spec.
Mito Tracker Red CMXRos	G1723-50UG	50 μg

Product Description/Introduction

MitoTracker Red CMXRos (Mitochondrial Red Fluorescent Probe) is an X-rosamine derivative with a molecular weight of 531.52, an excitation wavelength of 578 ± 3 nm and an emission wavelength of 599 ± 4 nm. The dye can directly enter living cells and locate on mitochondria and is able to covalently bind to mitochondria. The principle of specific labeling of mitochondria is that the probe skeleton with positive charge can be located on the negatively charged mitochondria, and the chloromethyl group on the probe can covalently bind to the free sulfhydryl group in the mitochondria , which plays the role of fluorescent labeling; the cell mitochondria can be labeled simply by incubating them with living cells for a certain period of time, and after labeling of the mitochondria, the mitochondria can be further processed through the fixation and perforation, which can be used for the subsequent multi-labeling experiments.

Storage and Shipping Conditions

Ship with wet ice; Store at -20℃ away from light, valid for 12 months.

Product Content

Component	G1723-50UG
Mito Tracker Red CMXRos	50 μg
Manual	1 pc

Assay Protocol / Procedures

1. Assay working solution preparation:

1.1. This product is in the form of a dry powder, which should be centrifuged briefly at high speed before use to ensure that it settles to the bottom of the tube. Provide your own cell grade DMSO.

1.2. Preparation of storage solution: Add 94 μL DMSO (cell level) into the tube, blow and mix well to fully dissolve the dye, that is to say, the mitochondrial fluorescent dye storage solution with a concentration of 1 mM (molecular weight information on the product label) is obtained, and then centrifuge briefly at a high speed to avoid the loss of reagents hanging on the wall. Store the storage solution at -20°C. It is recommended to store it in smaller sizes and avoid repeated freezing and thawing.

1.3. Preparation of assay working solution: If the assay is performed directly after staining, dilute the storage solution into mitochondrial assay working solution at a final concentration of 0.1-0.25 μM with biological buffer (Hanks, PBS) or serum-free basal medium; If subsequent operations such as fixation and perforation are required after staining, dilute the storage solution into mitochondrial assay working solution at a final concentration of 0.4-0.6 μM with biological buffer (Hanks, PBS) or serum-free basal medium;

2. Cell staining (the following protocol is for adherent cells; centrifugation is required for suspension cell exchange)

2.1. The cells are planted on cell culture plates or cell climbing plates until they converge to a certain extent;

2.2. The cell culture medium is removed and washed 1-2 times with preheated buffer for 3-5 min each time;

2.3. Add the preheated working solution for mitochondrial detection and incubate at 37°C for 30 min in a cell culture incubator (due to different cell types and states, the incubation time can be adjusted to some extent);

2.4. Remove the incubation solution and wash with buffer solution 2-3 times for 3-5 min each time;

2.5. Directly or with an Anti-fade Mounting Medium (recommended G1401) the films are sealed and observed under a fluorescence microscope (Ex=579 nm, Em=599 nm);

3. Cell fixation and perforation and other manipulations (optional):

Note: After staining, this product can be fixed and perforated, but the fluorescent signal will be attenuated after fixation.

3.1. Cell fixation

3.1.1. Cells treated with mitochondrial fluorescent dye labeling staining and washing are added to fixative (recommended G1101) and fixed for 10 min at room temperature;

3.1.2. Remove the fixative and wash with buffer 2-3 times for 3-5 min each time;

3.2. Cell perforation

3.2.1. The fixed and washed cells are added with 0.2-0.5% Triton X-100 and perforated for 10 min at room temperature;

3.2.2. The perforating fluid is removed and washed with buffer solution 2-3 times for 3-5 min each time;

3.3. The cells are stained and then fixed and perforated for processing, and can be used for the next experiments such as multilabeling.

Note

1. Due to the different types and states of the cells, the working concentration of the dye and the incubation time can be adjusted appropriately.
2. The dye needs to be used for mitochondrial localization in the state of living cells, and the cells cannot be fixed first, otherwise it will lead to false positive results of inaccurate localization. Fixation and subsequent operations can be performed after staining of living cells.
3. When staining and washing live cells, the assay working solution and washing buffer need to be pre-warmed at 37°C. Staining and washing are accomplished by incubation in an incubator so as not to affect the morphology of the cells by temperature difference stimulation.
4. In order to prevent fluorescence quenching, the entire staining process needs to be operated away from light.
5. Probe master mixes should be stored in separate containers to avoid repeated freezing and thawing.To avoid waste, the staining solution is prepared as needed, ready to use.
6. For your health and safety, please wear lab coat and gloves during operation.

Servicebio® Calcein AM

Cat. No.: G1728

Product Information

Product Name	Cat. No.	Spec..
Calcein AM	G1728-0.1ML	0.1 mL

Product Description/Introduction

Calcein AM (Calcein acetoxymethyl ester), is based on Calcein with the introduction of acetoxymethyl ester (AM) group, which increases the hydrophobicity, so it can easily penetrate the membrane of the living cells. Calcein AM itself is a cell-permeable, non-fluorescent and hydrophobic compound that, upon entry into the cell, is hydrolyzed by endogenous esterases in the cell to produce the strongly negatively charged polar molecule Calcein that cannot permeate the cell membrane, and thus is retained within the cell, whereas Calcein (maximum excitation wavelength: 494 nm; maximum emission wavelength: 517 nm) can emit strong green fluorescence. Compared to other similar probes (e.g. BCECF AM and CFDA AM), Calcein AM is currently one of the most ideal fluorescent probes for staining live cells due to its very low cytotoxicity, virtually no effect on cellular functions such as cell proliferation or lymphocyte chemotaxis, and low pH sensitivity.

Due to the lack of esterase in dead cells, Calcein AM is only used for viability testing and short-term labeling of living cells. The red fluorescent nucleic acid dye Propidium Iodide (PI) does not penetrate the cell membrane of living cells, it passes through the disordered region of the dead cell membrane to the nucleus and embeds itself in the cell's DNA double helix to produce a red fluorescence (excitation: 535 nm, emission: 617 nm), therefore PI stains only dead cells. Calcein AM is often used in combination with propidium iodide （PI） for simultaneous dual fluorescence staining of live and dead cells. Since both Calcein and PI-DNA can be excited at 490 nm, live and dead cells can be observed simultaneously by fluorescence microscopy. With 545 nm excitation, only dead cells can be observed.

Calcein AM can be used in most mammalian cells. It has been shown that Calcein AM can be used in some plant cells such as Arabidopsis root margin-like cells and some yeasts such as Pichia anomala and Saccharomyces cerevisiae. Certain parasites such as Leishmania cannot enter living cells due to cell membrane components, but Calcein AM can enter parasite cells in the early stages of apoptosis, and thus is used in conjunction with PI for the detection of parasites in the early stages of apoptosis. Calcein AM is not suitable for use on fungi and bacteria because they have cell walls that prevent Calcein AM from entering cells.

Calcein itself is a metal complexation indicator, and the fluorescent signal is quenched when it complexes with metal ions such as Co^{2+}, Ni^{2+}, Cu^{2+}, Fe^{3+}, and Mn^{2+} at physiological pH, making Calcein AM also a green fluorescent probe that can be used to determine the mitochondrial permeability transition pore.

This product, Calcein AM, is of high purity and dissolved in anhydrous DMSO at a concentration of 2 mM.The commonly used final concentration of Calcein AM is 0.1-5.0 μM.In order to obtain a more desirable result, please adjust the final concentration of Calcein appropriately according to the type of cells and the actual situation of the experiment.

Storage and Shipping Conditions

-20℃ for storage and transportation, valid for 12 months.

Product Content

Component	G1728-0.1ML
Calcein AM	0.1 mL
Manual	1 pc

Assay Protocol / Procedures

Calcein AM staining working solution was prepared:

Dilute the product with a suitable buffer, such as serum-free culture medium, HBSS (G4203), or PBS (G4202), to prepare a Calcein AM staining working solution at a concentration of 0.1-5.0 μM.

Note: The final concentration of Calcein AM is recommended to be adjusted appropriately according to the cell type and experimental reality.

Procedures

1. **Fluorescence microscopy assay or fluorescence zymography assay for suspension cells:**
   a) Cells were counted after certain treatments according to the experimental design. Take the appropriate cells and centrifuge them at 250×g for 5 min at room temperature, discard the supernatant, and add the appropriate volume of Calcein AM staining working solution to make the cell density about 1×10^6/ml.
   b) Incubate the cells at 37ºC for 30-45 min, the optimal incubation time is different for different cells. Consider 30 min as the initial incubation time, and optimize it according to the specific experimental situation to get more ideal detection results.
   c) At the end of incubation, centrifuge at 250×g for 5 min, aspirate the supernatant, and slowly resuspend the cells by adding 37ºC pre-warmed culture medium.
   d) Repeat step c two or more times to adequately wash to remove residual staining solution.
   e) Replace the fresh 37ºC pre-warmed culture medium and incubate at 37ºC for another 30 minutes away from light to ensure that the intracellular esterases sufficiently hydrolyze Calcein AM to produce Calcein with green fluorescence.
   f) The cells were centrifuged at 250×g for 5 min at room temperature, most of the culture medium was aspirated, and the cells were resuspended with the remaining culture medium and smeared, and then observed under a fluorescence microscope or detected by a fluorescence zymography.The maximal excitation wavelength of Calcein is 494 nm, and the maximal emission wavelength is 514 nm.If necessary, other fluorescence re-staining can be carried out, and the whole process should be avoided by light operation.
2. **Fluorescence microscopy assay of adherent cells or fluorescence enzyme labeling assay:**
   a) Cells were inoculated on petri dishes, multiwell cell culture plates or cell crawlers and treated in certain ways according to the experimental design.
   b) Aspirate the culture solution and wash the cells 1-2 times with PBS.
   c) Add an appropriate volume of Calcein AM Staining Solution and shake gently so that the dye covers all cells evenly. Generally, the volume of 96-well plate is 100 μl per well, 24-well plate is 250 μl per well, 12-well plate is 500 μl per well, and 6-well plate is 1 ml per well.
   d) Incubate at 37ºC for 30-45 min, the optimal incubation time varies for different cells. Take 30 min as the initial incubation time, and optimize the incubation time according to the cells used to get more ideal staining effect.
   e) At the end of the incubation, the culture medium was replaced with fresh pre-warmed culture medium at 37ºC and incubated at 37ºC for another 30 min away from light to ensure that the intracellular esterase sufficiently hydrolyzed Calcein AM to produce Calcein with green fluorescence.

f) Aspirate the culture fluid, wash with PBS for 2-3 times, and then add the serum-free cell culture fluid can be observed under a fluorescence microscope or detected by fluorescent enzyme marker.Calcein has a maximum excitation wavelength of 494 nm and a maximum emission wavelength of 514 nm, and can be further stained with other fluorescents if desired, keeping the entire process away from light.

3. Flow cytometry assay:

a) After trypsin digestion of adherent cells, resuspend the cells in culture medium, suspend the cells for direct use, count, centrifuge an appropriate amount of cells at 250×g for 5 min at room temperature, discard the supernatant, and add an appropriate volume of Calcein AM staining working solution so that the cells are a single-cell suspension and the density of the cells is approximately 1×106 /ml, with a volume of 1 ml for each sample. NOTE: It is necessary to prepare a buffer-only sample of the cells for use as a negative control in the flow cytometry assay. negative control for the flow cytometry assay, and it is desirable to keep this buffer the same as the buffer used to prepare the Calcein AM Staining Working Solution.

b) Incubate for 30 min at 37ºC away from light.

c) After the incubation was completed, cells were collected by centrifugation at 250 × g for 5 min at room temperature. Add 1 ml of buffer per sample, gently resuspend, and collect the cells by centrifugation at 250×g for 5 min at room temperature. Note: This step removes excess dye and reagents that may cause fluorescence quenching.

d) Resuspend cells with 400 μl buffer. Further staining can also be performed if required. Note that the entire process should be done away from light. After staining, the samples are placed on ice and can be flow cytometrically detected and analyzed within 1 hour.

e) Note that buffer-only and unstained cell samples were used for the negative control setting of the flow cytometer. Calcein has a maximum excitation wavelength of 494 nm and a maximum emission wavelength of 514 nm.

Note

1. All fluorescent dyes have quenching problems, and care must be taken to avoid light as much as possible to slow down fluorescence quenching.
2. Calcein AM is very sensitive to humidity and tends to decompose in humid environments, therefore Calcein AM solution must be tightly sealed with the cap after each withdrawal. It is recommended to store in separate sealed packages according to the single dosage. Do not use water-containing pipette tips.
3. Since Calcein AM is not stable in aqueous solutions such as PBS, the staining working solution must be prepared and used now.
4. Serum and phenol red in the culture medium have an effect on Calcein AM staining, and it is recommended that the cells be washed sufficiently before adding Calcein AM working solution.
5. Calcein AM labeled cells with uniform fluorescence are a good choice for cell tracking and general cytoplasmic staining, however, it does not bind to anything and may be actively withdrawn from the cell within a few hours, and the retention of calcein within living cells depends on the inherent characteristics of the cell type and culture conditions.
6. Calcein AM staining should not be followed by aldehyde fixation or Calcein will be lost during fixation. In addition, any disturbance of the plasma membrane (e.g., descaling agents or trypsin treatment) will result in dye leakage from the cells.
7. If Calcein AM has difficulty entering the cells, a surfactant such as Pluronic F127 can be used . A solution of Calcein AM at a concentration of 1/10 can also be used instead of culture medium.
8. This product is restricted to scientific research use by professionals, and is not to be used for diagnostic procedures or treatment, food or medicine, or stored in an ordinary residence.
9. For your safety and health, please wear lab coat and disposable gloves.

Servicebio® iF488-Wheat Germ Agglutinin（WGA，Green Light）

Cat. No.：G1730

Product Information

Product Name	Cat. No.	Spec.
iF488-Wheat Germ Agglutinin（WGA，Green Light）	G1730	100UL

Product Description/Introduction

Wheat germ agglutinin (WGA) is a lectin that binds to N-acetyl-D-glucosamine and sialic acid, and is one of the most studied and widely used classes of lectins. It is currently the most studied and widely used class of lectins.WGA binds to glycoconjugates, and its derivatives and conjugates are widely used for labeling cell membranes and fibrotic scar tissues for fluorescence imaging and analysis.The carbohydrate-binding specificity of WGA is directed towards a sequence of β-1,4-GlcNAc-linked residues, known as chitin dextrins. Each monosaccharide contains two identical, noninteracting binding sites that are complementary to three or four β-1,4-GlcNAc units. Of the monosaccharides tested, only GlcNAc binds to WGA, ManNAc does not, and GalNAc binds only weakly.WGA binds with high affinity to internal GlcNAc residues in large oligosaccharides containing Gal β(1-4)GlcNAc β(1-3) (i.e., poly(aminolactose)-type glycans) repeats.N-acetylneuraminic acid participates in the low-affinity interaction of WGA only. WGA shows a complex pattern of glycan specificity that can be used for structural analysis of complex carbohydrates.The iFluor®488 affix of WGA is probably the brightest WGA affix. It exhibits the bright and green fluorescence of the iFluor®488 dye. iFluor®488 WGA affix binds to sialic acid and N-acetylglucosaminyl residues as does the AF488 WGA coupler.

Wheat Germ Agglutinin (WGA) is a 36 kDa lectin commonly used to label mammalian cells, cell membranes of Gram-positive bacteria and yeast, as well as skeletal and cardiac sacral membranes, among others, due to its property of coupling to N-acetyl-β-D-glucosaminyl residues and N-acetyl-β-D-glucosaminyl oligomers in cell membranes. When WGA is used for cardiomyocyte staining, the cell membranes of cardiomyocytes can be stained out so that whether they are hypertrophic or not can be seen from the comparison of the image with the normal group, and the diameter and area of the stained cells can also be measured with specialized software, enabling analysis of whether the cardiomyocytes are hypertrophic or not.

Storage and Shipping Conditions

Ship with wet ice; Store at -20℃ for 12 months.

Product Content

Component	G1722-50UL
iF488-Wheat Germ Agglutinin (WGA, Green Light)	100 μL
Manual	1 pc

Pre-experiment preparation

1. Prepare your own **1×PBS buffer** (pH 7.2-7.4, recommended **G4202**), fixative containing 3.0-4.0% formaldehyde (recommended **G1101, containing 4% PFA**), **anti-fluorescence quenching sealer** (recommended **G1401**), and **ready-to-use DAPI staining solution** (recommended **G1012**).
2. When using the product for the first time, centrifuge the fully melted product at low speed for 1 min to prevent loss of liquid tube wall. It is recommended to dispense the product according to the amount used in a single experiment to prevent the loss of solvent by volatilization. Store at -20℃ away from light.
3. Before formal experiments, aspirate 5 μL iF488-Wheat Germ Agglutinin (WGA, Green Light) and mix with 1 mL PBS to get **iF488-WGA working solution**. The staining effect may be different for different types of cells, please refer to the literature for the optimal staining working concentration or conduct pre-test to find out. **iF488-WGA working solution** is ready to use, stored at room temperature and protected from light, and used on the same day.

Assay Protocol / Procedures

Live cell staining (12-well plate as an example)

1. Wash the cells with 1×PBS buffer for 2 times.
2. Add 100 μL of iF488-WGA working solution to each well of cells and incubate for 10-30 min at 37°C, protected from light. Take care of sealing to prevent liquid evaporation. Remove the incubation solution and wash with buffer solution 2-3 times for 3-5 min each time.
3. After the incubation was completed, the cells were washed three times with 1× PBS buffer buffer for 5 min each time.
4. Results were observed by fluorescence microscopy or laser confocal microscopy.

Fixed cell staining (6-well plate cell crawler as an example)

1. Cultured cells were crawled (at a density of at least 50% confluence), the culture medium was removed, and the cells were washed twice with 1× PBS buffer pre-warmed at 37°C.
2. Add appropriate amount of fixative to cover the cells and fix for 10-30 min at room temperature.
3. The fixative was aspirated and the cells were washed with 1× PBS buffer at room temperature 2-3 times for 10 min each.
4. After the cell crawler was slightly shaken dry, draw a circle with a histochemical pen so that the cells were located in the center of the circle. Take 100 μL of iF488-WGA working solution to cover the cells completely and incubate at 37°C for 30 min away from light. Be careful to seal and prevent the liquid from evaporating to dry the slides.
5. After the incubation was completed, the cells were washed three times with 1× PBS buffer for 5 min each time.
6. (Optional) Drops of ready-to-use DAPI staining solution were added to the crawler slides to cover the cells, and nuclear staining was performed for 8 min. The cells were washed with 1× PBS buffer 2-3 times for 30 s-1 min each time.
7. Cell crawls were slightly shaken dry and inverted onto a slide with a drop of anti-fluorescence quenching sealer, and excess sealer was blotted off with a paper towel.

   [Note**]** Steps 6 and 7, can be replaced with DAPI-containing anti-fluorescence quenching sealer

(Recommendation G1407), which accomplishes staining of the nuclei and sealing of the film at the same time.

8. The results were observed by fluorescence microscopy or laser confocal microscopy with 488 (Ex/Em=493/517 nm) and DAPI (Ex/Em=364/454 nm) channels selected.

Staining of tissue sections

1. Tissue sectioning pre-treatment:

   Frozen sections (fresh tissue frozen sections or fixed tissue frozen sections) were left to stand at room temperature for several minutes and returned to room temperature; sections were submerged in fixative for 10-15 min at room temperature, removed and dried naturally, and then moistened and washed in purified water or 1×PBS buffer to remove the residual fixative on the tissue.

   Paraffin sections were dewaxed and rehydrated;

   Tissue sections (paraffin sections or frozen sections of fixed tissues) were placed in a repair cassette filled with EDTA antigen repair buffer (pH 8.0) in a microwave oven for antigen repair. Medium heat for 8 min cease fire for 8 min to medium-low heat for 7 min, this process should prevent excessive evaporation of buffer, do not dry the slides. After natural cooling, the slides were placed in 1×PBS buffer and washed by shaking on a decolorizing shaker for 3 times, each time for 5 min.

2. Staining: Draw a circle around the tissue with a histochemical pen after the tissue section is slightly dried. Add 50-100 μL of iF488-WGA working solution to the circle to cover the tissue, and incubate at 37℃ away from light for 30 min. Take care of sealing to prevent the liquid from evaporating and drying the slides.
3. Wash: After the incubation was completed, the samples were washed three times with 1× PBS buffer for 5 min each time.
4. Nucleus staining (optional): dropwise add ready-to-use DAPI staining solution to the sample to cover the cells, and perform nucleus staining for 8 min. Wash the sample with 1×PBS buffer 2-3 times for 30 s-1 min each time.
5. Sealing: After the sections are slightly shaken dry, add a drop of Anti-fluorescence quenching sealer to the sample and seal it with a suitable coverslip.

   [Note**]** Steps 4 and 5, can be replaced with DAPI-containing anti-fluorescence quenching sealer (Recommendation G1407), which accomplishes staining of the nucleus and sealing of the film at the same time.
6. Microscopy: Fluorescence microscopy or laser confocal microscopy to observe the results, selecting the 488 (Ex/Em=493/517 nm) and DAPI (Ex/Em=364/454 nm) channels.

Note

1. Samples that have been fixed for a long period of time (e.g., paraffin sections) must be subjected to a pyrolysis repair process or else the positive results will be weak or close to none.
2. WGA staining for myocardial analysis requires a high level of myocardial tissue, which must be complete and homogeneous, and it is best to provide specimens of paraffin sections.
3. For cellular samples, avoid the use of permeabilizing solutions prior to staining, as this may cause staining of cytoplasmic structural components.
4. Fluorescent dyes are subject to quenching, and it is recommended that testing and observation photos be completed as soon as possible after staining.
5. Please wear lab coat and gloves during operation.

Servicebio® iF555-Wheat Germ Agglutinin（WGA，Red Light）

Cat. No.：G1731

Product Information

Product Name	Cat. No.	Spec.
iF555-Wheat Germ Agglutinin（WGA，Red Light）	G1731	100UL

Product Description/Introduction

Wheat germ agglutinin (WGA) is a lectin that binds to N-acetyl-D-glucosamine and sialic acid, and is one of the most studied and widely used classes of lectins. It is currently the most studied and widely used class of lectins.WGA binds to glycoconjugates, and its derivatives and conjugates are widely used for labeling cell membranes and fibrotic scar tissues for fluorescence imaging and analysis.The carbohydrate-binding specificity of WGA is directed towards a sequence of β-1,4-GlcNAc-linked residues, known as chitin dextrins. Each monosaccharide contains two identical, noninteracting binding sites that are complementary to three or four β-1,4-GlcNAc units. Of the monosaccharides tested, only GlcNAc binds to WGA, ManNAc does not, and GalNAc binds only weakly.WGA binds with high affinity to internal GlcNAc residues in large oligosaccharides containing Gal β(1-4)GlcNAc β(1-3) (i.e.,poly(aminolactose)-type glycans) repeats.N-acetylneuraminic acid participates in the low-affinity interaction of WGA only. WGA shows a complex pattern of glycan specificity that can be used for structural analysis of complex carbohydrates.The iFluor®555 affix of WGA is probably the brightest WGA affix. It exhibits the bright and red fluorescence of the iFluor®555 dye. iFluor®555 WGA affix binds to sialic acid and N-acetylglucosaminyl residues as does the AF555 WGA coupler.

Wheat Germ Agglutinin (WGA) is a 36 kDa lectin commonly used to label mammalian cells, cell membranes of Gram-positive bacteria and yeast, as well as skeletal and cardiac sacral membranes, among others, due to its property of coupling to N-acetyl-β-D-glucosaminyl residues and N-acetyl-β-D-glucosaminyl oligomers in cell membranes. When WGA is used for cardiomyocyte staining, the cell membranes of cardiomyocytes can be stained out so that whether they are hypertrophic or not can be seen from the comparison of the image with the normal group, and the diameter and area of the stained cells can also be measured with specialized software, enabling analysis of whether the cardiomyocytes are hypertrophic or not.

Storage and Shipping Conditions

Ship with wet ice; Store at -20℃ for 12 months.

Product Content

Component	G1722-50UL
iF555-Wheat Germ Agglutinin（WGA，Red Light）	100 μL
Manual	1 pc

Pre-experiment preparation

1. Prepare your own **1×PBS buffer** (pH 7.2-7.4, recommended **G4202**), fixative containing 3.0-4.0% formaldehyde (recommended **G1101, containing 4% PFA**), **anti-fluorescence quenching sealer** (recommended **G1401**), and **ready-to-use DAPI staining solution** (recommended **G1012**).
2. When using the product for the first time, centrifuge the fully melted product at low speed for 1 min to prevent loss of liquid tube wall. It is recommended to dispense the product according to the amount used in a single experiment to prevent the loss of solvent by volatilization. Store at -20℃ away from

light.

3. Before formal experiments, aspirate 5 μL iF555-Wheat Germ Agglutinin (WGA, Red Light) and mix with 1 mL PBS to get **iF555-WGA working solution**. The staining effect may be different for different types of cells, please refer to the literature for the optimal staining working concentration or conduct pre-test to find out. **iF555-WGA working solution** is ready to use, stored at room temperature and protected from light, and used on the same day.

Assay Protocol / Procedures

Live cell staining (12-well plate as an example)

1. Wash the cells with 1×PBS buffer for 2 times.
2. Add 100 μL of iF555-WGA working solution to each well of cells and incubate for 10-30 min at 37°C, protected from light. Take care of sealing to prevent liquid evaporation. Remove the incubation solution and wash with buffer solution 2-3 times for 3-5 min each time.
3. After the incubation was completed, the cells were washed three times with 1× PBS buffer buffer for 5 min each time.
4. Results were observed by fluorescence microscopy or laser confocal microscopy.

Fixed cell staining (6-well plate cell crawler as an example)

1. Cultured cells were crawled (at a density of at least 50% confluence), the culture medium was removed, and the cells were washed twice with 1× PBS buffer pre-warmed at 37°C.
2. Add appropriate amount of fixative to cover the cells and fix for 10-30 min at room temperature.
3. The fixative was aspirated and the cells were washed with 1× PBS buffer at room temperature 2-3 times for 10 min each.
4. After the cell crawler was slightly shaken dry, draw a circle with a histochemical pen so that the cells were located in the center of the circle. Take 100 μL of iF555-WGA working solution to cover the cells completely and incubate at 37°C for 30 min away from light. Be careful to seal and prevent the liquid from evaporating to dry the slides.
5. After the incubation was completed, the cells were washed three times with 1× PBS buffer for 5 min each time.
6. (Optional) Drops of ready-to-use DAPI staining solution were added to the crawler slides to cover the cells, and nuclear staining was performed for 8 min. The cells were washed with 1× PBS buffer 2-3 times for 30 s-1 min each time.
7. Cell crawls were slightly shaken dry and inverted onto a slide with a drop of anti-fluorescence quenching sealer, and excess sealer was blotted off with a paper towel.

   [Note**]** Steps 6 and 7, can be replaced with DAPI-containing anti-fluorescence quenching sealer (Recommendation G1407), which accomplishes staining of the nuclei and sealing of the film at the same time.
8. The results were observed by fluorescence microscopy or laser confocal microscopy with 555 (Ex/Em=556/574 nm) and DAPI (Ex/Em=364/454 nm) channels selected.

Staining of tissue sections

1. Tissue sectioning pre-treatment:

   Frozen sections (fresh tissue frozen sections or fixed tissue frozen sections) were left to stand at room temperature for several minutes and returned to room temperature; sections were submerged in fixative for 10-15 min at room temperature, removed and dried naturally, and then moistened and

washed in purified water or 1×PBS buffer to remove the residual fixative on the tissue.

Paraffin sections were dewaxed and rehydrated;

Tissue sections (paraffin sections or frozen sections of fixed tissues) were placed in a repair cassette filled with EDTA antigen repair buffer (pH 8.0) in a microwave oven for antigen repair. Medium heat for 8 min cease fire for 8 min to medium-low heat for 7 min, this process should prevent excessive evaporation of buffer, do not dry the slides. After natural cooling, the slides were placed in 1×PBS buffer and washed by shaking on a decolorizing shaker for 3 times, each time for 5 min.

2. Staining: Draw a circle around the tissue with a histochemical pen after the tissue section is slightly dried. Add 50-100 μL of iF555-WGA working solution to the circle to cover the tissue, and incubate at 37℃ away from light for 30 min. Take care of sealing to prevent the liquid from evaporating and drying the slides.
3. Wash: After the incubation was completed, the samples were washed three times with 1× PBS buffer for 5 min each time.
4. Nucleus staining (optional): dropwise add ready-to-use DAPI staining solution to the sample to cover the cells, and perform nucleus staining for 8 min. Wash the sample with 1×PBS buffer 2-3 times for 30 s-1 min each time.
5. Sealing: After the sections are slightly shaken dry, add a drop of Anti-fluorescence quenching sealer to the sample and seal it with a suitable coverslip.

   [Note**]** Steps 4 and 5, can be replaced with DAPI-containing anti-fluorescence quenching sealer (Recommendation G1407), which accomplishes staining of the nucleus and sealing of the film at the same time.
6. Microscopy: Fluorescence microscopy or laser confocal microscopy to observe the results, selecting the 488 (Ex/Em=556/574 nm) and DAPI (Ex/Em=364/454 nm) channels.

Note

1. Samples that have been fixed for a long period of time (e.g., paraffin sections) must be subjected to a pyrolysis repair process or else the positive results will be weak or close to none.
2. WGA staining for myocardial analysis requires a high level of myocardial tissue, which must be complete and homogeneous, and it is best to provide specimens of paraffin sections.
3. For cellular samples, avoid the use of permeabilizing solutions prior to staining, as this may cause staining of cytoplasmic structural components.
4. Fluorescent dyes are subject to quenching, and it is recommended that testing and observation photos be completed as soon as possible after staining.
5. Please wear lab coat and gloves during operation.

Servicebio® Lipid Droplets Fluorescence Assay Kit

Cat. No.: G1905

Product Information

Product Name	Cat. No.	Spec.
Lipid Droplets Fluorescence Assay Kit	G1905-100T	100 T

Product Description/Introduction

Lipid droplets are specialized lipid storage organelles in cells, which can regulate the storage and hydrolysis of neutral lipid in cells and provide dynamic energy balance for cells. In addition, lipid droplets are able to move along the cytoskeleton and interact with other organelles, playing an important role in the regulation of signal transduction. Abnormal accumulation of cellular lipid droplets occurs in several types of metabolic diseases, such as obesity, fatty liver, cardiovascular diseases, and pathological states.

Lipid Droplet Fluorescence Assay Kit is based on BODIPY 493/503, which is a lipophilic fluorescent probe capable of localizing to polar lipids, and can be used to calibrate the neutral lipid content of cells and tissues.This kit has been optimized and debugged by our R&D team, and has multiple advantages such as easy to use, stable performance and good reproducibility, etc. The higher the intracellular lipid content, the stronger the intensity of fluorescence generated, which can be detected by fluorescence microscope and flow cytometer. In addition, this kit additionally provides oleic acid as an inducer of triglyceride synthesis and storage, which can be used for positive control induction.

Storage and Shipping Conditions

Ship with dry ice; Store at -20℃ away from light for 6 months.

Product Content

Component Number	Component	G1904-100T
G1905-1	Lipid droplet assay probe (2 mM)	100 μL
G1905-2	Lipid Droplet Assay Buffer	100 mL
G1905-3	Positive inducer (500 mM)	50 μL
Manual		1 pc

Note: The above reaction times are assayed for a 6-well plate system.

Assay Protocol / Procedures

Note: Take 6-well plate adherent cells as an example, suspension cells need to be centrifuged every time the liquid is changed; Tissue samples directly refer to the staining and labeling steps, and the overall volume of reagents used is adjusted according to the situation; If necessary, the samples can be fixed in advance, but please avoid perforation of the samples.

1. Preparation of working solution for lipid droplet assay and working solution for lipid droplet induction:
   1.1. Take appropriate amount of lipid droplet assay probe (2 mM) and lipid droplet assay buffer, mix thoroughly at a ratio of 1 : 500-2000, and prepare a working solution for lipid droplet assay;
   1.2. Take an appropriate amount of positive inducer (500 mM) and use the cell culture medium of the corresponding test cells to dilute it to 400-1000 uM, and prepare a lipid droplet induction working solution for spare use (this concentration is a reference range, and can be adjusted appropriately according to the nature of the cells or other circumstances).
2. Positive control group and drug and other treatments (6-well plate adherent cells, for example,

suspension cells each time the change of liquid, please perform centrifugation):

2.1. Cells are pre-planted in 6-well plates as needed (with or without crawler depending on assay and equipment);

2.2. Remove the original cell culture medium and wash twice with PBS or other buffers;

2.2.1. Experimental group: the cells to be tested are subjected to drug stimulation or other relevant treatments, and the desired incubation time is set by yourself;

2.2.2. Positive control group: 1 mL of lipid droplet induction working solution is added to the well plate, which is placed in an incubator for overnight incubation;

2.3. At the end of the incubation, turn to step 3 for stain labeling.

3. Lipid droplet staining marker:

3.1. Remove the cellogenic medium and wash 2-3 times with PBS or other buffers;

3.2. Remove the wash buffer, add 1 mL of Lipid Droplet Assay Working Solution and mix with gentle shaking;

3.3. Incubate for 15 min in a CO_2 incubator protected from light;

3.4. After incubation, wash it twice with PBS or other buffers, and it can be further stained with nuclei and other operations or detected with the appropriate equipment according to the experimental needs;

4. Lipid droplet detection:

4.1. The sample after incubation and washed in 3.4 is analyzed using the appropriate method and instrumentation, labeled to show green fluorescence, EX/EM 493/503 nm, and some examples of instrumental detection are given below;

4.1.1. Directly or with anti-fluorescence quenching sealing solution (Item No. G1401), the film is sealed and then placed under a fluorescence microscope for detection and analysis.

4.1.2. After digestion and resuspension using tryptic digest (suspension cells are not used), cells are detected and analyzed using flow cytometry.

Note

1. Due to the different types and properties of cells, the concentration of positive inducers and probes and the incubation time can be adjusted appropriately according to the needs of the experiment.
2. Samples should avoid contact with organic solvents or other components that affect lipids to avoid interfering with the accuracy of the assay.
3. The fluorescent dyes are subject to quenching, and the process should be protected from light.
4. For your health and safety, please wear lab coat and gloves during operation.

Servicebio® Aging Cell β-galactosidase Staining Kit

Cat No.: G1073-100T

Product Information

Product Name	Cat.No.	Spec.
Aging Cell β-galactosidase Staining Kit	G1073-100T	100 T

Description

The division ability of most normal cells is limited. When they cannot divide, they enter a state of senescence, which is called cell senescence. Cell senescence is the guarantee mechanism for a cell to control its growth potential, which generally means replicative senescence. Normal cells stop dividing after a limited number of divisions, and irreversible growth arrest occurs. At this time, the cells are still alive, but there are significant changes in cell morphology and physiological metabolic activity, usually represented by larger cell volume and activation of β-galactosidase associated with aging. β-galactosidase is a hydrolytic enzyme in cell lysosomes. It is usually active at pH 4.0, but it is active at pH 6.0 in senescent cells. This kit is based on this phenomenon and principle to stain aging tissues or cells against the up-regulation of β-galactosidase activity level associated with aging. The specific reaction principle is that X-Gal is used as the substrate, and senescent cell specific β-galactosidase catalyzes the substrate to generate blue product, which is represented by blue sediment in the cytoplasm of the cell, which can be observed under the light microscope. According to the calculation that the amount of staining solution for each sample is 1 mL, the kit can complete the staining of 100 samples.

Storage and Handling Conditions

Wet ice transportation; Store at 2-8℃ away from light, valid for 12 months. If X-Gal powder is prepared into a solution, it is divided into small parts and stored at -20℃, which is effective within 3 months.

Component

Component Number	Component	G1073
G1073-1	β-galactosidase Staining Fixation Solution	100 mL
G1073-2	β-galactosidase Staining Solution A	100 mL
G1073-3	β-galactosidase Stain B	1.2 mL
G1073-4	DMF (Dimethylformamide)	5 mL
G1073-5	X-Gal (Powder)	100 mg

Assay Protocol

I Preparation of reagents

1. Prepare your own PBS buffer (**G4202 recommended**).

2. 100 mg X-Gal powder was fully dissolved and mixed with 5 mL DMF (dimethylformamide), and then divided into 1.5 mL clean centrifuge tubes, 0.5 mL for each tube, and stored at -20℃ away from light. Avoid repeated freezing and thawing.

3. Preparation of β-galactosidase staining solution according to the proportion in the table below. For cells cultured in 6-well plates, 1.0-1.5 mL of staining working solution is required per well, and for 12-well plates, 0.5-1.0 mL of staining working solution is required per well. The staining solution was prepared according to the sample size to avoid waste.

Component	Volume
β-galactosidase staining solution A	940 μL
β-galactosidase stain B	10 μL
X - Gal solution	50 μL
Total Volume	1 mL

I Staining procedure

1. For adherent cells

(1) The cultured cells (or cell crawling sheets) in 6-well plates were aspirated and the cell culture medium was removed, washed twice with PBS, and 1 mL β-galactosidase staining fixing solution was added, and the cells were fixed for 15 min at room temperature.

(2) The fixed solution was discarded, and the cells were washed with PBS for 3 times, 2 min each time.

(3) PBS was removed by suction with a pipette, and 1 mL of β-galactosidase staining working solution was added to each well and incubated at 37℃ for 2 h to overnight. Note: Do not incubate in carbon dioxide incubator at 37°C. During the staining period, the color development should be observed in time. If the expression of β-galactosidase in the sample is high, the staining can be completed within a few hours. If β-galactosidase expression was low, the incubation time should be extended appropriately, during which the 6-well plate should be sealed with plastic wrap or parafilm to prevent liquid evaporation from affecting the staining results.

(4) Under the ordinary light microscope, the staining solution was removed after the positive cells developed color. If nuclei need to be counterstained, add a small amount of Nuclear Fast Red solution (**G1035 is recommended**) to the well plate to cover the cells and stain at room temperature for 3 min, remove the staining solution, and wash with PBS several times.

(5) 2 mL PBS was added to cover the cells and the staining was completed. The sample could be stored at 4℃ for 1 week. Or add 70% glycerol to cover the cells, 4℃ can be stored for a long time. If it is the cell climbing sheet, the climbing sheet can be fully dried, xylene transparent after dropping neutral gum seal sheet, can be stored for a long time.

2. For frozen sections

(1) Rewarm frozen sections at room temperature for 10 min. Circle the tissue with tissue strokes.

(2) A proper amount of β-galactosidase staining fixing solution was added to the tissue to completely cover the tissue, and the solution was fixed at room temperature for 20 min.

(3) The tissue sections were soaked and washed in PBS for 3 times, 5 min each time.

(4) The sections were placed in a wet box to avoid light, and an appropriate amount of β-galactosidase staining solution was added to the tissue to completely cover the tissue. The wet box was incubated at 37℃ and the color development was observed under a microscope every 2 h. If no color development was

observed, the culture was continued until the senescent cells on the tissue showed color. If the sample is to be incubated overnight, a sufficient amount of β-galactosidase staining solution should be added to prevent the staining solution from evaporating and drying the tablets.

(5) After the tissue developed color, the staining solution was removed, and the sections were immersed in PBS and washed twice, and then immersed in pure water and washed twice.

(6) (optional) Add Nuclear Fast Red solution (**G1035 is recommended**) for 3 min and wash for 3 times.

(7) The sections were dehydrated with absolute ethanol for 2 times, then transparent with xylene for 5 min each time, and then sealed with neutral gum drop.

3. Staining results

The cytoplasm of senescent cells is scattered blue.

Note:

1. X-Gal solution should be thawed and mixed completely at room temperature before use.
2. β-galactosidase staining solution A and B should be restored to room temperature in advance before use, and the prepared staining solution should be thoroughly mixed without precipitation before use.
3. The β-galactosidase staining reaction of senescent cells is dependent on specific pH conditions, so it cannot be incubated in a CO_2 incubator for color development, otherwise it will affect the pH of the staining solution and lead to staining failure.
4. When preparing dyeing solution, please choose consumables made of polypropylene (PP) or glass instead of polystyrene (PS).
5. The color development should be observed several times during the 2 h-overnight color development period, too short a time may lead to negative results; too much time can lead to false positives. The chromogenic time is closely related to the amount of β-galactosidase contained in the sample itself.
6. Before preparing the staining solution, check the pH value of staining solution A. If it is not 6.0 (which may be changed due to storage conditions), adjust the pH value to 6.0 with HCl or NaOH before use.
7. β-galactosidase staining of tissue sections requires high preparation of samples, which should be stored at -80℃ and tested as soon as possible. Because β-galactosidase is very easy to inactivate, improper storage or too long of the sample may lead to enzyme inactivation, then no positive staining.
8. Please wear a lab coat and disposable gloves during operation

Images:

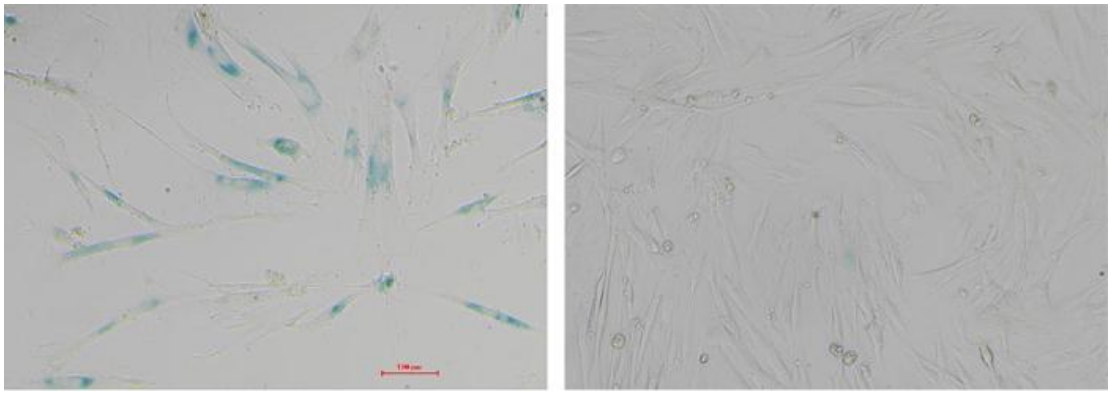


Fig.1 WI-38 cells were stained with β-galactosidase kit. The left picture shows senescence WI-38 cells without division and proliferation ability but still alive. After staining, the positive staining cells were more than 95%. The image on the right shows newly resuscitated WI-38 cells (early passage) with less than 3 passages, and no obvious positive cells after staining.

Servicebio® Senescence associated β-gal-actosidase,SA-β-GAL

Cat. No.: G1125

Product Information

Product Name	Cat. No.	Spec..
Senescence associated β-gal-actosidase,SA-β-GAL	G 1125-100ML	100 mL

Product Description/Introduction

This product is a β-galactosidase fixative solution for aging cell β-galactosidase staining. The vast majority of positive normal cells have a limited ability to divide and then enter the senescence state after they fail to divide. This process is called cell senescence, which is fine cell senescence is a mechanism by which cells control their growth potential. It's replicative senescence. Normal cell meridian After a finite number of divisions, the division stops and irreversible growth arrest occurs. At this time, the cell is still alive, but the cell morphology and physiological generation. There is a significant change in the activity of the cell, which is usually characterized by increased cell size and activation of β-galactosidase associated with aging. Beta galactosidase Lysozyme is a hydrolytic enzyme in cellular lysozyme, usually active at pH 4.0, but active at pH 6.0 in senescent cells. Failure β-galactosidase fixation solution of old cells can fix β-galactosidase in senescent cells.

The main ingredients of this product are 0.01M phosphate buffer, 2% formaldehyde, 0.2% glutaraldehyde.

Storage and Shipping Conditions

Transport at room temperature; Store at 4℃ away from light, valid for 12 months

Product Content

Component Number	Component	G
G1125	Senescence associated β-gal-actosidase, SA-β-GAL	100mL

Assay Protocol / Procedures

1. For adherent cells: Cells cultured in 6-well plates (or cell slides) were sucked off the cell culture medium, washed twice with PBS, and 1 mL was added senescent cells β-galactosidase fixative solution was fixed at room temperature for 15 min, then the fixative solution was removed, and the senescent cells β was cleaned with PBS buffer solution galactosidase staining.
2. For frozen section: rewarm the frozen section at room temperature for 10 min. Circle the tissue with a circular stroke. An appropriate amount of senescent cells were dripped onto the tissue β-galactosidase fixating solution was fixed for 20 min at room temperature to cover the tissue completely. Tissue sections were soaked and washed with PBS β-galactosidase staining of senescent cells.

Note

1. Please use it in a well-ventilated environment and take good protection to avoid inhalation.
2. After use, please tighten the bottle cap in time to prevent volatilization of active ingredients.
3. For your safety and health, please wear a lab coat and disposable gloves

Servicebio® Dual-Lumi Dual-Luciferase Reporter Gene Assay Kit

Cat. No.: G1701-100T

Product Information

Product Name	Cat. No.	Spec.
Dual-Lumi Dual-Luciferase Reporter Gene Assay Kit	G1701-100T	100T

Product Description/Introduction

Luciferase system is mostly used for reporter gene detection. The transcriptional regulatory elements of the target gene are cloned into the upstream and downstream of Luciferase to construct a reporter gene plasmid. The transfected cells are then treated with drugs or other stimuli, and then the cells are collected and lysed, and the luciferase activity in the lysate is measured. The transcriptional regulation effect of exogenous stimuli, such as drugs, on target genes is determined by luciferase activity. In the dual fluorescence system, two kinds of fluorescence are introduced and detected without interfering with each other, one is used as the detection fluorescence, and the other is used as the internal reference fluorescence to eliminate the differences in the number of cells and the transfection efficiency.

This Dual-Lumi Dual Luciferase (Firefly&Ranilla Luciferase) Reporter Gene Assay Kit is designed to detect firefly luciferase activity by using firefly luciferin as a substrate first, and then detect ranilla luciferase activity by using coelenterazine as a substrate while quenching firefly fluorescence. The kit is characterized by rapid detection, high sensitivity, wide detection range, and the two kinds of fluorescence detection do not interfere with each other.

Storage and Shipping Conditions

Ship with dry ice; Store at -80 ℃ away from light for 12 months; -20 ℃ store away from light, recommended for use within 6 months.

Product Content

Component Number	Component	G1701-100T
G1701-1	Cell Lysis Buffer	50 mL
G1701-2	Firefly Luciferase Reaction Substrate (50×)	200 μL
G1701-3	Firefly Luciferase Reaction Buffer	10 mL
G1701-4	Ranilla Luciferase Reaction Substrate (50×)	200 μL
G1701-5	Ranilla Luciferase Reaction Buffer	10 mL
Manual		1 pc

Assay Protocol / Procedures

1. Prepare 1× Firefly Luciferase Reaction working solution:

1.1. The kit is separated and thawed at room temperature and mixed upside down to ensure complete dissolution of the components. The Firefly Luciferase Reaction Substrate (50×) and Ranilla Luciferase Reaction Substrate (50×) should be thawed on ice and centrifuged for a short period of time to ensure that the reagents sink to the bottom of the tube;

1.2. Dilute the firefly luciferase reaction substrate (50×) 50 fold with firefly luciferase reaction buffer, i.e., 10 μL of firefly luciferase reaction substrate (50×) and 490 μL of firefly luciferase reaction buffer are mixed well to obtain 500 μL of 1× firefly luciferase reaction working solution for spare use;

1.3. The Ranilla Luciferase Reaction Substrate (50×) is diluted 50-fold with Ranilla Luciferase Reaction Buffer, i.e., 10 μL of Ranilla Luciferase Reaction Substrate (50×) is mixed with 490 μL of Ranilla Luciferase Reaction Buffer to obtain 500 μL of 1× sea kidney luciferase reaction working solution. Ranilla Luciferase Reaction Buffer mixed well to obtain 500 μL of 1× Ranilla Luciferase Reaction Substrate (50×);

Note: Prepare according to the dosage to avoid waste. It is recommended that the luciferase reaction working solution be used now and stored at -20℃ for use within one month to avoid repeated freezing and thawing.

2. Cell pretreatment and lysis:

2.1. Cells are implanted in the corresponding plates, and transfected or other related pre-treatments according to the experimental needs;

2.2. For adherent cells: after removing the original cell culture medium, add the corresponding amount of **Cell Lysis Buffer** according to the table below to cover the cells;For suspended cells, transfer the cells into a centrifuge tube, centrifuge to remove the medium, and add the corresponding amount of **Cell Lysis Buffer** to resuspend the cells according to the table below;

Cell Culture Plate	Cell Lysis Buffer/well
6-well	500 μL
12-well	300 μL
24-well	200 μL
48-well	150 μL
96-well	100 μL

2.3. After sufficient lysis for 10-20 min at room temperature, cells are scraped and collected in a 1.5 mL centrifuge tube, centrifuged at 4°C, 12000 g for 10 min, and the supernatant (cell lysate) is retained for spare use;

3. Fluorescence detection:

3.1. Pre-prepared 1× firefly luciferase reaction working solution and 1× Ranilla luciferase reaction working solution are equilibrated to room temperature;

3.2. 1× Firefly Luciferase Reaction Work Solution at 100 μL/well is added to an opaque white 96-well plate;

3.3. The cell lysis supernatant from step 2 is added to the 96-well plate above at 20 μL/well;

3.4. The horizontal oscillation is uniform, as soon as possible, use Luminometer, multifunctional enzyme marker with chemiluminescence detection module or other instruments that can detect bioluminescence, chemiluminescence detection -- detection of firefly luciferase reporter gene activity (fluorescence intensity);

3.5. 1× Ranilla Luciferase Reaction Work Solution at 100 μL/well is added to the wells where firefly luciferase reporter gene activity has been detected as described above;

3.6. The horizontal oscillation is uniform, as soon as possible, use Luminometer, multifunctional enzyme marker with chemiluminescence detection module or other instruments that can detect bioluminescence, chemiluminescence detection -- detection of Ranilla luciferase reporter gene activity (fluorescence intensity).

Note

1. Before detection, the 1× Firefly Luciferase Reaction working solution needs to be restored to room temperature before use.

2. Luciferase Reaction Buffer has some precipitation after thawing, which is a normal phenomenon, and it should be shaken well before use to ensure that it is completely dissolved.
3. 1×Luciferase reaction working solution is susceptible to oxidation, when conducting multi-sample detection, the addition of 1× Luciferase Reaction working solution should be controlled within a short period of time as far as possible, It is recommended to use multichannel pipette for sample addition and pay attention to whether the suction volume of each well of the pipette is consistent.
4. In order to prevent interference between wells, the use of opaque white well plates is recommended.
5. For your health and safety, please wear lab coat and gloves during operation.

Servicebio® Mono-Lumi Firefly Luciferase Reporter Gene Assay Kit

Cat. #：G1702-100T

Product Information

Product Name	Cat. No.	Spec.
Mono-Lumi Firefly Luciferase Reporter Gene Assay Kit	G1702-100T	100T

Product Description/Introduction

The firefly-luciferase system is mostly used for reporter gene detection. The transcriptional regulatory elements of the target gene were cloned upstream and downstream of the luciferase to construct a reporter gene plasmid. The plasmid was then transfected into cells, and after treating the transfected cells with drugs or other stimuli, the cells were collected and lysed, and luciferase activity in the lysate was measured. The transcriptional regulation of target genes by exogenous stimuli such as drugs is determined by luciferase activity.

The Mono-Lumi Firefly Luciferase Reporter Gene Assay Kit has high sensitivity, broad detection range and stable fluorescence signal. Using Firefly Luciferase, which catalyzes the oxidation of firefly luciferin substrate (luciferin) to generate oxyluciferin under the conditions of Mg^{2+}, ATP, O_2, etc. and generates biofluorescence in the process, the principle of Firefly Luciferase activity can be rapidly detected.

Storage and Shipping Conditions

Ship with dry ice; store at -80℃ protected from light, valid for at least one year; store at -20℃ protected from light, for up to 6 months.

Product Contents

Component Number	Component	G1702-100T
G1702-1	Cell Lysis Buffer	50 mL
G1702-2	Luciferase Reaction Substrate (50×)	200 μL
G1702-3	Luciferase Reaction Buffer	10 mL
Manual		One copy

Assay Protocol / Procedures

1. Preparation of 1× firefly luciferase reaction working solution

1.1 Thaw each component of the kit at room temperature and mix upside down to ensure complete dissolution. The Luciferase Reaction Substrate (50×) should be thawed on ice and centrifuged briefly to ensure that the reagent sinks to the bottom of the tube;

1.2 The Luciferase Reaction Substrate (50×) was diluted 50 times with Luciferase Reaction Buffer, i.e., 10 μL Luciferase Reaction Substrate (50×) + 490 μL Luciferase Reaction Buffer was mixed well to obtain 500 μL of 1× Firefly Luciferase Reaction Buffer.

2. Cell pretreatment and lysis

2.1 Plate cells/well in appropriate well plates, perform cell transfection or other relevant pretreatment according to the experimental requirement.

2.2 For adherent cells: After removing the original cell culture medium, add appropriate amount of Cell

Lysis Buffer to each well according to the table below.

For suspension cells: After centrifuge, remove the supernatant and add appropriate amount of Cell Lysis Buffer to precipitated cells

Refer to the table below for the amount of lysis buffer suggested .

Cell Culture well-Plate	Cell Lysis Buffer/Well
6-well	500 μL
12-well	300 μL
24-well	200 μL
48-well	150 μL
96-well	100 μL

2.3 Lysis cells completely at room temperature for 10-20 min, collect cells in a 1.5mL centrifuge tube by centrifuge at 12,000 ×g at 4℃ for 10 min, keep the supernatant (cell lysate) for later use.

3. Firefly luciferase assay

3.1 Pre-prepared 1× firefly luciferase reaction working solution was equilibrated to room temperature and added to an opaque white 96-well plate at 100 μL/well;

3.2 The cell lysis supernatant from step 2 was added to the above 96-well plate at 20 μL/well;

3.3 Shake horizontally to homogeneity and immediately perform a chemiluminescence assay using a Luminometer luminescence meter, a multifunctional enzyme labeler with a chemiluminescence detection module, or other instrument capable of detecting bioluminescence.

Note

1. Prior to the assay, the pre-prepared 1× Firefly Luciferase Reaction Work Solution needs to be brought back to room temperature before use.
2. Luciferase Reaction Buffer may precipitate after thawing. Please shake thoroughly before use to ensure complete dissolution.
3. The 1× luciferase reaction working solution is prone to be oxidized. For multi-sample detection, the addition of 1× firefly luciferase reaction working solution should be controlled in a short time. It is recommended to add samples with the multichannel pipettes, and keep the volume of each well is consistent.
4. To prevent interference between wells, it is recommended to use a white, opaque well plate. The black, opaque well plate can also be used, but it will absorb the fluorescence signal, reducing the fluorescence signal of detection.
5. For your safty and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® Mono-Lumi Ranilla Luciferase Reporter Gene Assay Kit

Cat. No.: G1703-100T

Product Information

Product Name	Cat. No.	Spec.
Mono-Lumi Ranilla Luciferase Reporter Gene Assay Kit	G1703-100T	100T

Product Description/Introduction

Luciferase system is mostly used for reporter gene detection. The transcriptional regulatory elements of the target gene are cloned into the upstream and downstream of Luciferase to construct a reporter gene plasmid. The plasmid is then transfected into cells, and after treating the transfected cells with drugs or other stimulants, the cells are collected and lysed, and the luciferase activity in the lysate is measured. The transcription regulation of target genes effect of exogenous stimuli such as drugs is determined by luciferase activity.

Mono-Lumi Ranilla Luciferase Reporter Gene Assay Kit, using Ranilla Luciferase, catalyzes the oxidation of Coelenterazine substrate to Coelenteramide in the presence of oxygen, and generates biofluorescence in the process, The principle that the fluorescence intensity generated is proportional to the luciferin activity can be rapidly detected. The kits are characterized by rapid detection, high sensitivity and wide detection range.

Storage and Shipping Conditions

Ship with dry ice; Store at -80 ℃ away from light for 12 months; -20 ℃ store away from light, recommended for use within 6 months.

Product Content

Component Number	Component	G1703-100T
G1703-1	Cell Lysis Buffer	50 mL
G1703-2	Ranilla Luciferase Reaction Substrate (50×)	200 μL
G1703-3	Ranilla Luciferase Reaction Buffer	10 mL
Manual		1 pc

Assay Protocol / Procedures

1. **Prepare 1× Ranilla Luciferase Reaction working solution:**
   1.1. The kit is thawed at room temperature, and mixed upside down to ensure that each component is completely dissolved. The Ranilla Luciferase Reaction Substrate (50×) should be thawed on ice and centrifuged briefly to ensure that the reagent sinks to the bottom of the tube;
   1.2. The Ranilla Luciferase Reaction Substrate (50×) is diluted 50-fold with Ranilla Luciferase Reaction Buffer, i.e., 10 μL Ranilla Luciferase Reaction Substrate (50×) is mixed with 490 μL Ranilla Luciferase Reaction Buffer to obtain 500 μL of 1× Ranilla Luciferase Reaction working solution;

   Note: Prepare according to dosage to avoid waste. 1× Ranilla Luciferase Reaction working solution is recommended to be prepared and used now.
2. **Cell pretreatment and lysis:**
   2.1. Cells are implanted in the corresponding plates, and transfected or other related pre-treatments according to the experimental needs;

2.2. For adherent cells: after removing the original cell culture medium, add the corresponding amount of **Cell Lysis Buffer** according to the table below to cover the cells;For suspended cells, transfer the cells into a centrifuge tube, centrifuge to remove the medium, and add the corresponding amount of **Cell Lysis Buffer** to resuspend the cells according to the table below;

Cell Culture Plate	Cell Lysis Buffer/well
6-well	500 μL
12-well	300 μL
24-well	200 μL
48-well	150 μL
96-well	100 μL

2.3. After sufficient lysis for 10-20 min at room temperature, cells are scraped and collected in a 1.5 mL centrifuge tube, centrifuged at 4°C, 12000 g for 10 min, and the supernatant (cell lysate) is retained for spare use;

3. Ranilla Luciferase detection:

3.1. 1× Ranilla Luciferase Reaction working solution prepared in advance is restored to room temperature and added to an opaque white 96-well plate at 100 μL/well;

3.2. The cell lysis supernatant retained in step 2 is added to the above 96-well plate at 20 μL/well;

3.3. The horizontal oscillation is uniform, and the chemiluminescence detection is performed immediately with the Luminometer, the multifunctional enzyme label with the chemiluminescence detection module or other instruments capable of detecting bioluminescence,

Note

1. Before detection, the 1× Ranilla Luciferase Reaction working solution needs to be restored to room temperature before use.
2. Ranilla Luciferase Reaction Buffer has some precipitation after thawing, which is a normal phenomenon, and it should be shaken well before use to ensure that it is completely dissolved.
3. When conducting multi-sample detection, the addition of 1× Ranilla Luciferase Reaction working solution should be controlled within a short period of time as far as possible, It is recommended to use multichannel pipette for sample addition and pay attention to whether the suction volume of each well of the pipette is consistent.
4. In order to prevent interference between wells, the use of opaque white well plates is recommended. Opaque black plates can also be used, but black will absorb the fluorescence signal and reduce the fluorescence signal detected.
5. For your health and safety, please wear lab coat and gloves during operation.

Servicebio® Reactive Oxygen Species (ROS) Assay Kit

Cat. #：G1706-100T

Product Information

Product Name	Cat. No	Spec.
Reactive Oxygen Species (ROS) Assay Kit	G1706-100T	100T

Product Description/Introduction

Reactive oxygen species (ROS) Assay Kit (ROS Assay Kit), is a Kit for ROS detection based on the fluorescent probe DCFH-DA. DCFH-DA (2',7' -dichlorofluorescein diacetate, MW 487.29), is not fluorescent itself and can freely cross the cell membrane. Once entering cells, DCFH can be hydrolyzed by esterase, which is widely existed in cells. DCFH is not permeable to cell membrane and retained by cells DCFH without fluorescence can be oxidized by intracellular reactive oxygen species (ROS) to generate fluorescent DCF, and the intensity of fluorescence signal generated is proportional to the level of intracellular ROS. The level of intracellular reactive oxygen species can be indirectly evaluated by fluorescence microscopy, flow cytometry or laser confocal microscopy.

Reactive oxygen species (ROS) Assay kit can effectively detect intracellular ROS with high sensitivity and easy to use. In the kit, Reactive Oxygen Positive Inducer is also provided, which can increase ROS levels in multiple types of cells in a short time. Based on the standard of sampling amount per well of 6-well plate, this kit can be determined about 100 times.

Storage and Shipping Conditions

Ship with wet ice; store at -20℃ away from light, valid for 12 months.

Product Contents

Component Number	Component	G1706-100T
G1706-1	DCFH-DA probe	100 μL
G1706-2	Reactive Oxygen Positive Inducer (2×)	20 mL
Manual		One copy

Assay Protocol / Procedures

1. Preparation before experiment:

1.1. Preparation of DCFH-DA working solution: Dilute DCFH-DA probe with serum-free cell medium or Earle's balanced salt solution (G4213) at a ratio of 1:1000.

1.2. (Optional) Preparation of 1×ROS positive inducer working solution: Dilute ROS positive inducer (2×) to 1 × working solution with serum-free cell medium or Earle's balanced salt solution (G4213).

2. (Optional) Cell preparation for positive control group:

2.1. Remove cells from growth media via centrifugation or pipetting.

2.2. Resuspend cells in 1×ROS positive inducer working solution.

2.3. Incubate for 45 min in a 37°C incubator with CO2, protected from light. (Note: The sensitivity of different cells is different. If the increase of reactive oxygen species is not detected after 45 minutes of , the induction time can be appropriately prolonged or the concentration of inducer can be increased; if the increase of reactive oxygen species is too fast, the induction time or the concentration of inducer can be appropriately decreased).

3. Reactive oxygen species detection of adherent cells:

3.1. Cell planting: At least one day in advance, the cells in good condition are evenly planted into the well platewith a certain density and treated with drugs or other pretreatments according to the experimental needs. (The planting density is determined by factors such as cell size and growth rate, and ensure that the cell confluence is 50-70% during the test.)

3.2. Cell washing: Discard the cell culture medium and wash it with PBS buffer (G4202) 1-2 times to reduce the interference of serum, drugs and other substances on the experimental results:

3.3. Probe Loading: Discard the washing buffer, add the corresponding volume of DCFH-DA working solution according to the table below, and incubate in a CO_2 incubator at 37℃ for 30 min under light.

3.4. Discard DCFH-DA working solution and wash with PBS buffer 2-3 times to fully remove the excess probe.

3.5. Cover the cells with PBS buffer.

Cell Culture Plate	Reactive Oxygen Species Detection Working Solution/Well
6-well	1000 μL
12-well	500 μL
24-well	250 μL
48-well	200 μL
96-well	100 μL

3.4. ROS detection: The labeled cells in the well plate are detected by fluorescence microscope or confocal microscope. Or after digestion, the cells are collected and detected by fluorescence spectrophotometer, microplate reader, flow cytometer and other instruments. The fluorescence spectrum of DCF is very similar to that of FITC, the excitation wavelength of 488nm and emission wavelength of 525nm,, and the parameters of FITC can be set to detect DCF.

4. Detection of reactive oxygen species in suspension cells (also applicable to adherent cells resuspended after digestion and pretreatment):

2.1. Cell pretreatment: Select cells in good condition according to the experimental requirements, and centrifuge them at 1,000 rpm for 3-5 min after drug or other pretreatment according to the experimental requirements to collect cells (cell density shall be determined according to the subsequent detection system, method and total amount, for example, the number of cells in a single sample by flow detection shall be no less than 10^4);

2.2. Cell washing: Wash 1-2 times with PBS buffer, centrifuge at 1000 rpm for 3-5 min to remove supernatant and collect cells;

2.3. Probe loading: Add a certain volume of DCFH-DA working solution to make the cell density of $1x10^5$-$5x10^5$ cells /mL, and incubate in a CO_2 incubator at 37℃ for 30 min in the dark. During the period, gently shake the mixture every 5 min to ensure full contact between the probe and the cell. At the end of the incubation, the DCFH-DA working solution is removed by centrifugation at 1,000 rpm for 3-5 min and washed 2-3 times with PBS buffer to adequately remove excess probes. Finally, the cells are resuspended in PBS.

2.4. ROS detection: Cells labeled with probe can be directly detected by fluorescence spectrophotometer, microplate reader, flow cytometer, etc. It can also be dropped onto a slide and observed with instruments such as fluorescence or confocal microscopy. With the excitation wavelength of 488nm and emission wavelength of 525nm, the fluorescence spectrum of DCF is very similar to that of FITC, and the parameters of FITC can be set to detect DCF.

Note

1. If the overall fluorescence intensity after probe labeling is too high or too low during the experiment, the dilution ratio and incubation time of DCFH-DA probe can be adjusted appropriately.
2. If the probe does not enter the cell, it will cause a high background. Please clean it as far as possible.
3. The positive inducer of reactive oxygen species is mainly used for the induction of positive control samples. According to the experimental needs, whether to be a positive control or not.
4. Each centrifugation of cells will cause a certain loss to the total cell density. Please pay attention to control the initial cell amount to prevent the final cell amount from being insufficient for detection.
5. Due to quenching of fluorescent probe, please perform ROS detection as soon as possible after probe incubation.
6. For your safty and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® Fluo-4 AM Fluorescent Calcium Ion Detection Kit

Cat. No.: G1724

Product Information

Product Name	Cat. No.	Spec.
Fluo-4 AM Fluorescent Calcium Ion Detection Kit	G1724-100T	100 T

Product Description/Introduction

Fluo-4 is an upgraded version of Fluo-3 commonly used in biological calcium ion detection experiments, the main difference is that the two chlorine atoms on the Fluo-3AM molecule are replaced by fluorine on Fluo-4AM, and the small change in the structure can make Fluo-4AM load faster and the detection signal is stronger at the same concentration. Because the AM group of Fluo-4AM probe not only covers the molecular part of Fluo-4 chelated calcium but also enhances its hydrophobicity, it can easily penetrate the living cell membrane and enter the cell. When Fluo-4AM enters the cell, it will be clipped into Fluo-4 by lactase, thus remaining in the cell. Fluo-4 itself has little fluorescence, but when combined with intracellular calcium ions, it can produce strong fluorescence. The fluorescence intensity is proportional to the intracellular calcium content.

This kit is mainly based on the above detection principles, Fluo-4AM as the main body, and provides a special matching buffer, easy to use, more sensitive detection. The content of calcium ions in living cells or the dynamic change of calcium ions in cells can be measured by fluorescence microscope, flow analyzer, enzyme labeler, etc.

Storage and Shipping Conditions

Ship with dry ice; Store at -20℃ away from light, valid for 12 months.

Product Content

Component Number	Component	G1724-100T
G1724-1	Fluo-4 AM fluorescent probe	20 μL
G1724-2	Calcium ion detection buffer	20 mL
Manual		1 pc

Note: The above kit reaction times correspond to the 96-well plate assay system.

Assay Protocol / Procedures

1. **Pre-experiment preparation:** Cells are inoculated in 96-well plates and pre-treated accordingly to the experimental purpose;
2. **Configuration of Fluo-4 AM working solution**: Prepare Fluo-4 AM working solution according to the need, the configuration system can be referred to the following table, note that different cell characteristics are different, according to the actual situation of the experiment can be added or reduced the concentration of the probe used, the initial recommended dilution ratio of 1:500;

	1 sample	10 samples	100 samples
Fluo-4 AM fluorescent probe	0.2 μL	2 μL	20 μL
Calcium ion detection buffer	100 μL	1 mL	10 mL

3. **Staining markers** (with adherent cells as an example; suspension cell need fluid change every time, so please perform centrifugation by yourself):

3.1. Remove the original cell culture medium and wash the cells 2-3 times with PBS or other buffers;

3.2. Remove the washing buffer, add 100μL Fluo-4 AM working solution to each well, and incubate in a cell incubator at 37℃ for 30min away from light;

3.3. Remove the Fluo-4 AM working solution at the end of the incubation and wash the cells 2-3 times using PBS or other buffers;

3.4. Replace with calcium ion detection buffer and continue incubation for 15-30min to ensure adequate hydrolysis of Fluo-4AM in cells (optional);

4. **Fluorescence detection**: Use fluorescence microscope, enzyme marker and other instruments to carry out its detection. Fluo-4 AM is green fluorescent with EX/EM ≈ 494/528 nm.

Note

1. Due to different intercellular properties and different pre-treatment methods, the ratio of probe use and incubation time range described in the steps are for reference only and can be adjusted according to specific circumstances.
2. Fluorescent dyes have quenching problems, please pay attention to avoid the light operation to slow down the fluorescence quenching.
3. Fluo-4AM is very sensitive to humidity, so please pack it properly according to the experimental arrangement when using it for the first time. The Fluo-4 AM Assay Working Solution should be prepared ready to use.
4. Some precipitation after thawing is a normal phenomenon, and it is fully shaken before use to ensure that it is completely dissolved.
5. Based on the working principle of this probe, it is not possible to treat the cells such as fixation or perforation.
6. For your health and safety, please wear lab coat and gloves during operation.

Servicebio® MQAE Fluorescent Chloride Ion Detection Kit

Cat. No.: G1725

Product Information

Product Name	Cat. No.	Spec.
MQAE Fluorescent Chloride Ion Detection Kit	G1725-100T	100 T

Product Description/Introduction

MQAE is a 6-methoxyquinolinium derivative. Compared with the chloride fluorescence probe SPQ, MQAE has higher chloride sensitivity and fluorescence quantum yield, and it is the most widely used new chloride fluorescence probe. The probe can detect intracellular chloride ions by diffusion-restricted collision quenching. When intracellular chloride ion concentration increases, the fluorescence intensity of MQAE decreases proportionally with the increase of chloride ion concentration.

This kit is based on the above detection principles, mainly MQAE, and provides a special supporting buffer, easy to use, sensitive detection. The dynamic changes of chloride ions in cells can be detected by fluorescence microscopy, flow cytometry or enzyme labeling.

Storage and Shipping Conditions

Ship with dry ice; Store at -20℃ away from light for 12 months.

Product Content

Component Number	Component	G1725-100T
G1725-1	MQAE fluorescent probe	100 μL
G1725-2	Chloride Detection Buffer	20 mL
Manual		1 pc

Note: The above kit reaction times correspond to the 96-well plate assay system.

Assay Protocol / Procedures

1. **Pre-experiment preparation:** Cells are inoculated in 96-well plates and pre-treated accordingly to the experimental purpose;
2. **Configuration of MQAE working solution**: Configure MQAE working solution according to the need, the configuration system can refer to the following table. Note that different cell characteristics are different. During the experiment, the concentration of the probe can be added or reduced according to the actual situation, initial recommended dilution ratio at 1:100;

	1 sample	10 samples	100 samples
MQAE fluorescent probe	1 μL	10 μL	100 μL
Chloride Detection Buffer	100 μL	1 mL	10 mL

3. **Staining markers** (with adherent cells as an example; suspension cell treatment requires centrifugation):
   - 3.1. Remove the original cell culture medium and wash the cells 1-2 times with PBS or other buffers;
   - 3.2. Remove the washing buffer, add 100 μL of MQAE working solution to each well, and incubate for 30 min at 37°C in a cell culture incubator protected from light;
   - 3.3. Remove the MQAE working solution at the end of the incubation and wash the cells 1-2 times using PBS or other buffers;

4. **Fluorescence detection**: Use fluorescence microscope, enzyme marker and other instruments to carry out its detection. The probe is green fluorescent, EX/EM ≈ 319/462 nm.

Note

1. Due to different intercellular properties and different pre-treatment methods, the ratio of probe use and incubation time range described in the steps are for reference only and can be adjusted according to specific circumstances.
2. Fluorescent dyes have quenching problems, please pay attention to avoid the light operation to slow down the fluorescence quenching.
3. The probe is sensitive to humidity. When used for the first time, please pack it properly to avoid repeated freezing and thawing. MQAE working solution should be dispensed ready to use.
4. For your health and safety, please wear lab coat and gloves during operation.

Servicebio® BCECF, AM Fluorescence pH Assay Kit

Cat. No.: G1726

Product Information

Product Name	Cat. No.	Spec..
BCECF, AM Fluorescence pH Assay Kit	G1726-100T	100 T

Product Description/Introduction

Intracellular pH plays an important role in many cellular activities, such as cell growth, enzyme activity, ion transport, and other processes.BCECF, AM is a cell-permeable pH fluorescent dye that is widely used to monitor changes in intracellular pH by contrasting the ratio of dual excitation wavelengths. BCECF, AM is non-fluorescent and can be sheared into BCECF by intracellular esterases upon entry into the cell, thus being retained inside the cell.BCECF, AM has been widely used not only in mammalian cells, but also has been reported to be used for intracellular pH levels in animal tissues, plant cells, bacteria and yeasts.

This kit is based on the above detection principle, with BCECF, AM as the main body, and provides specialized matching buffer, which is easy to use and more sensitive to detect. It can be used to monitor the dynamic change of intracellular pH with fluorescence microscope, enzyme labeling instrument and other corresponding instruments.

Storage and Shipping Conditions

Transported on dry ice; stored dry and protected from light at -20°C. Valid for 12 months.

Product Content

Component Number	Component	G1726-100T
G1726-1	BCECF, AM fluorescent probe	20 μL
G1726-2	pH testing buffer	20 mL
Manual		1 pc

Note: The above kit reaction times correspond to the 96-well plate assay system.

Assay Protocol / Procedures

1. **Pre-experimental preparations:** Cells were inoculated in advance in 96-well plates at a certain density, and the cells were pre-treated accordingly according to the purpose of the experiment;
2. **BCECF, AM assay working solution preparation:** according to the need to prepare **BCECF, AM assay working solution**, the preparation system can be referred to the following table, note that different cell characteristics are different, according to the actual situation of the experiment can be added or reduced the concentration of the probe used, the initial recommendation of the dilution ratio of 1:500;

	1 sample	10 samples	100 samples
BCECF, AM fluorescent probe	0.2 μL	2 μL	20 μL
pH testing buffer	100 μL	1 mL	10 mL

3. **Staining markers** (in the case of adherent cells; suspension cells need to be centrifuged and changed each time):
   - 3.1 Remove the original cell culture medium and wash the cells 1-2 times with PBS or other buffers;
   - 3.2 Remove the washing buffer, add 100 μL of BCECF, AM assay working solution to each well, and incubate for 30 min at 37°C in a cell culture incubator protected from light;
   - 3.3 At the end of the incubation remove the BCECF, AM assay working solution and wash the cells

2-3 times using PBS or other buffers;

Note: Wash out the BCECF, AM assay working solution.

3.4 100 μL of buffer was added for fluorescence detection.

4. **pH calibration** (pH calibration by nigericin method as an example, optional):

4.1 Prepare buffers of different pH (e.g., 6.6, 7.0, 7.2, 7.4, 7.8, 8.2) in advance;

4.2 After staining and labeling, buffers of different pH as well as nigericin (final concentration of 10 μg/mL) were added respectively, and the fluorescence intensity was measured after incubation for 10 min at 37°C in a cell incubator protected from light;

4.3 Plotting of fluorescence intensity versus pH graphs for sample pH calculations.

Note: The nigericin method of calibrating pH is for reference only, you can choose the corresponding method according to your experimental needs. In general, the higher the fluorescence intensity ratio, the higher the pH.

5. **Fluorescence assay:** The intracellular pH level is detected and analyzed using an enzyme marker and other related instruments. Usually BCECF, AM is applied to the double excitation ratio: EX/EM=490/530 nm : EX/EM=440/530 nm.

Note

1. Due to the different intercellular properties and pre-treatments, the proportion of probes used and the range of incubation times described in the steps are for reference only and can be adjusted on a case-by-case basis.
2. Fluorescent dyes are subject to quenching, so please try to avoid light to slow down the fluorescence burst.
3. BCECF, AM is very sensitive to humidity, when you use it for the first time, please divide it appropriately according to the experimental arrangement, and avoid repeated freezing and thawing.BCECF, AM assay working solution should be prepared and used now.
4. Shake well before use to ensure complete dissolution.
5. Based on the working principle of this probe, cells cannot be fixed or perforated.
6. For your health and safety, please wear a lab coat and gloves when operating.

Servicebio® FerroOrange Fluorescent Method Ferrous Ion Detection Kit

Cat. No.: G1727

Product Information

Product Name	Cat. No.	Spec.
FerroOrange Fluorescent Method Ferrous Ion Detection Kit	G1727-100T	100 T

Product Description/Introduction

FerroOrange, also known as RhoNox-4, specifically detects intracellular free ferrous (II) ions (Fe^{2+}). The basic principle of the kit is that after FerroOrange reacts with ferrous ions, an orange fluorescence product is irreversibly generated, and its fluorescence intensity is proportional to the content of ferrous ions in the cell. Neither iron (III) ions (Fe^{3+}) nor divalent metal ions other than iron increase their fluorescence intensity. FerroOrange is highly permeable to the cell membrane and can remain in the cell after rapid reaction, so it is suitable for live cell imaging.

Based on the above detection principles, this kit takes FerroOrange as the main body, and provides special supporting buffer and positive inducer. Fluorescence microscope, enzyme labeling instrument and other corresponding instruments can be used to detect ferrous ions in living cells, which is convenient to use and sensitive to detect.

Storage and Shipping Conditions

Ship with dry ice; Store at -80℃ away from light for 6 months.

Product Content

Component Number	Component	G1724-100T
G1727-1	FerroOrange fluorescent probe	20 μL
G1727-2	Ferrous ion detection buffer	20 mL
G1727-3	Ferrous ion positive inducer	2×1 mL
Manual		1 pc

Note: The above kit reaction times correspond to the 96-well plate assay system.

Assay Protocol / Procedures

1. **Pre-experiment preparation:** Cells are inoculated in 96-well plates and pre-treated accordingly to the experimental purpose;
2. **Configuration of FerroOrange working solution**: Prepare FerroOrange working solution according to the need, the configuration system can be referred to the following table, note that different cell characteristics are different, according to the actual situation of the experiment can be added or reduced the concentration of the probe used, the initial recommended dilution ratio of 1:500; Before preparation, vortex and centrifuge the FerroOrange fluorescent probe briefly to ensure that all reagents are immersed in the bottom of the tube;

	Experimental group	Control group
FerroOrange fluorescent probe	0.2 μL	0.2 μL
Ferrous ion detection buffer	100 μL	90 μL
Ferrous ion positive inducer		10 μL

3. **Staining markers** (with adherent cells as an example;Suspension cell treatment requires centrifugation):
   3.1. Remove the original cell culture medium and wash the cells 1-2 times with PBS or other buffers;;
   3.2. Remove the washing buffer, add 100μL Fluo-4 AM working solution to each well, and incubate in a cell incubator at 37℃ for 30min away from light;
   3.3. Remove the FerroOrange working solution at the end of the incubation and wash the cells 1-2 times using PBS or other buffers;
   3.4. Add 100 μL PBS or other buffer solution to cover adherent cell, or resuspend cell pellet, for the next fluorescence detection;
4. **Fluorescence detection**: Use fluorescence microscope, enzyme marker and other instruments to carry out its detection. FerroOrange is orange fluorescent with EX/EM ≈ 543/580 nm. The fluorescence intensity of positive control group treated with positive inducer should be weaker than that of experimental group.

Note

1. Due to different intercellular properties and different pre-treatment methods, the ratio of probe use and incubation time range described in the steps are for reference only and can be adjusted according to specific circumstances.
2. Fluorescent dyes have quenching problems, please pay attention to avoid the light operation to slow down the fluorescence quenching.
3. FerroOrange is very sensitive to humidity, so please pack it properly according to the experimental arrangement when using it for the first time.
4. FerroOrange can only detect free bivalent iron and cannot detect bound iron.
5. The increase of fluorescence intensity after the reaction of FerroOrange with Fe^{2+} is irreversible and cannot be used for real-time dynamic monitoring of ferrous ion levels.
6. Based on the working principle of this probe, it is not possible to treat the cells such as fixation or perforation.
7. Some precipitation after thawing is a normal phenomenon, and it is fully shaken before use to ensure that it is completely dissolved.
8. For your health and safety, please wear lab coat and gloves during operation.

Dihydroethidium

Cat.No. : G1904-100T

Brand : Servicebio

Spec.: 100 T

Product Introduction

Product Information

Product Name	Cat. No.	Spec.
Dihydroethidium	G1904-100T	100 T

Product Description/Introduction

Dihydroethidium (DHE), is a commonly used fluorescent detection probe for superoxide anions. DHE freely enters the cell and dehydrogenates in the presence of intracellular superoxide anions to produce ethidium bromide. Ethidium bromide binds to intracellular nucleic acids and produces red fluorescence. When the intracellular superoxide anion level is high, the more ethidium bromide is produced, the higher the fluorescence intensity, and vice versa the weaker the fluorescence intensity. Therefore, it can be used to detect the level of intracellular superoxide anion.

Storage and Shipping Conditions

Ship with dry ice; Store at -20℃ away from light for 6 months.

Product Content

Component	G1904-100T
Dihydroethidium	100 μL
Manual	1 pc

Note: The above reaction counts were assayed for a 6-well plate system.

Assay Protocol / Procedures

1. This product is a 5 mM Dihydroethidium (superoxide anion fluorescent probe) masterbatch, which is diluted into a DHE working solution (usually 1-10 μM is sufficient for the experiment) using appropriate medium or buffer;
2. Wash normal or treated cells 1-2 times with PBS or other buffers;
3. After removing the washing buffer, add 1 mL of DHE working solution and incubate for 30 min in a cell culture incubator protected from light;
4. Remove the DHE working solution and wash 1-2 times with PBS or other buffers;
5. Test under the corresponding instrument; DHE itself is blue fluorescence, Ex is 355 nm, Em is 420 nm; When DHE dehydrogenates and binds to nucleic acid, it shows red fluorescence, Ex is 520 nm, Em is 605 nm.

Note

1. Due to the different types and properties of the cells, the working concentration of the probe and the incubation time can be adjusted appropriately according to the needs of the experiment.
2. Probe working solution, washing buffer, etc. need to be pre-warmed and processed in advance to avoid temperature difference stimulation affecting the cells.
3. The fluorescent dyes are subject to quenching, and the process should be protected from light.
4. Dihydroethidium is easily oxidized in air, avoid exposure to air as much as possible, it is recommended to store in small portions and avoid repeated freezing and thawing.
5. For your health and safety, please wear lab coat and gloves during operation.

Servicebio® Lentivirus Concentration Solution (5×)

Cat. #：G1801-100ML

Product Information

Product Name	Cat. No.	Spec.
Lentivirus Concentration Solution (5×)	G1801-100ML	100 mL

Product Description/Introduction

The Lentivirus Concentration Solution (5×) provides a fast and simple method for concentrating all lentiviral stocks. Concentration is achieved by mixing a lentiviral supernatant with this concentration reagent, followed by a short incubation step and centrifugation in a standard centrifuge. The recovery rate can reach more than 90% and the virus titer can be increased by 100 folds.

Storage and Shipping Conditions

Ship with wet ice; store at 2-8℃ for 12 months.

Product Contents

Component Number	Component	G1801-100 mL
G1801	Lentivirus Concentration Solution (5×)	100 mL
Manual		One copy

Assay Protocol / Procedures

1. Harvest the lentivirus-containing supernatants. Cells and debris were removed by filtration using sterile filters with 0.45 μm membranes (low protein uptake PES membranes are recommended). And the PES filter membrane should be pre-cooled with 3-5 mL PBS once before passing through the virus stock solution.)
2. The lentiviral supernatant and 5× lentiviral precipitation concentrate were mixed in an inverted ratio of 4:1 (4 parts of viral supernatant and 1 part of viral concentrate), and the mixture was incubated at 4°C for 2 h, during which time it was inverted up and down for 10 times at 30 min intervals for mixing, or left to be incubated at 4°C overnight (an appropriately prolonged incubation time can improve the virus recovery rate).
3. After incubation for a certain period of time, centrifuge at 4℃, 8000 g for 10~15 min.
4. Carefully remove the supernatant, resuspend the lentiviral particles with 1/10~1/100 of the original volume of DMEM or PBS (G4202 is recommended) (in some cases, the precipitate may not necessarily be visible to the naked eye), gently blow to homogeneity, after resuspension of the precipitate, you can determine the titer of the concentrated lentiviral samples and then dispense them according to the experimental use volume and place them at -80℃ for storage (try to minimize the number of times that the lentiviruses have been repeatedly frozen and thawed, so as not to reduce the titer of the virus).

Note

1. Filters and syringes are not provided with this kit. Please prepare your own filters and syringe.
2. Although the ingredients in this kit have a certain protective effect against lentivirus, please try to avoid lentivirus repeated freeze-thaw cycles.
3. For your safty and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® Rapid Lentivirus Titration Cassette (Colloidal Gold)

Cat. #: G1804

Product Information

Product Name	Cat. No.	Spec.
Rapid Lentivirus Titration Cassette (Colloidal Gold)	G1804-10T	10 T

Product Description

This product is a semi-quantitative HIV-1 P24 protein assay card based on colloidal gold lateral chromatography that can be used for rapid assessment of the packaging efficiency of relevant lentivirus vectors and pseudoviruses (the P24 protein is the most abundant marker protein in the lentiviral capsid). The product is a rapid detection of free capsid protein P24 secreted into the lentiviral packaging supernatant using colloidal gold immunochromatography as a sensitive, indirect surrogate indicator of viral yield. The detection of capsid protein P24 enables a rapid determination of whether the lentiviral titer in the packaging solution has met the harvest needs. This product requires only 8-12 minutes to obtain results, which is a significant advantage over traditional transfection methods that take 2-3 days.

P24 protein concentration in relation to lentivirus titer:

P24 concentration	500 ng/mL	100 ng/mL	50 ng/mL	10 ng/mL	1 ng/mL
LPs/mL	6.25×10^{9}	1.25×10^{9}	6.25×10^{8}	1.25×10^{8}	1.25×10^{7}
TU/mL	$6.25\times10^{6\text{-}7}$	$1.25\times10^{6\text{-}7}$	$6.25\times10^{5\text{-}6}$	$6.25\times10^{5\text{-}6}$	$6.25\times10^{4\text{-}5}$

There are approximately 2000 P24 protein molecules in a Lentiviral Particle (LP) and normally 1 TU (Transducing Unit) of infectious virus vector for every 100-1000 LPs; a virus titer of at least 1 x 10^{6} TU/mL is required for certain types of cells to be transfected. (usually concentrated at around 150 times).

Result of lentivirus titer fast detection card (colloidal gold method) for 10 min:

Dilute P24 protein to 500 ng/mL, 100 ng/mL, 50 ng/mL, 10 ng/mL, 1 ng/mL and 0, then drop 80 μ L of diluent into the loading wells, place it flat on the table, stand for 8-12 minutes to observe the results.

Storage and Shipping Conditions

Ship and store at room temperature, valid for 12 months.

Product Components

Component Number	Component	G1804-10T
G1804-10T	Rapid Lentivirus Titration Cassette (colloidal gold)	10 T
Manual		1 pc

Assay Protocol

1. Open the package and take out the detection card, place it flat on the table.
2. Pipette 80~100 μL of culture supernatant from cells (such as 293 cells) packaged with lentivirus, and drop to the loading wells.
3. Stand for 8~12 minutes to obtain results, and they are invalid after 15 minutes.

Result Judgment

1. In the visual window, both the quality control line (C) and the test line (T) are coloured as positive.
2. The quality control line (C) is colored, the test line (T) is not colored, and the test result is negative.
3. The quality control line (C) is not colored. No matter whether the detection line (T) is colored or not, the result is invalid.
4. The colour of the (T) line varies with the content of P24, the higher the content, the darker the colour; the lower the content, the lighter the colour.

Note

1. If the color of tissue culture medium causes high background, dilute the sample with PBS or culture medium before chromatography.
2. It is not recommended to freeze the test card.
3. Use as soon as possible after opening.

Servicebio® PEI 40K Transfection Reagent

Cat.#：G1802

Product information

product name	Identification of product	model
PEI 40K Transfection Reagent	G1802-1ML	1 mL
	G1802-10ML	10 mL

Descirption/Introduction

Transfection of DNA into eukaryotic cells is a common method to study biological mechanisms. A major goal is the efficient and specific delivery of genes into the desired target cells. Although a wide panel of techniques and vectors (viral and nonviral) have been developed that work with variable efficiency, most vectors lack a target cell specificity. Nonviral vectors are attractive because of their ease of manipulation, safety, and high flexibility in the size of the delivered transgene. The Transferrinfection is a high-efficiency nucleic acid delivery system based on transferrin receptor-mediated endocytosis to carry DNA into cells. Furthermore it was shown that the cationic polymer polyethylenimine (PEI) mediates efficient gene transfer into a variety of cells. In the Polyethylenimine-Transferrinfection system, the gene transfer efficiency of PEI/DNA complexes are combined with the specific mechanism of receptor-mediated endocytosis via Transferrin receptor.

The method results in a 30–1,000-fold enhanced transfection efficiency depending on the cell line. It is an extremely gentle DNA transfection method that employs physiological uptake mechanisms of the cell. Transfection efficacy depends on the cell type, the level of surface transferrin receptor expression. Very high transfection rates have been shown for the tested tumor cell lines B16F10 melanoma, Neuro 2A neuroblastoma, and a variety of primary human melanoma cell lines. In other established cell lines, such as HeLa, CHO, Jurkat, K562, HepG2, and COS, the PEI-Transferrinfection works with high efficiences, excellent reproducibility and with the advantage of being an extremely gentle procedure.

Human transferrin is covalently linked to a polycationic carrier (Polyethylenimine/PEI) with intrinsic/ inherent endosomal activity. PEI has the capacity to condense DNA and deliver it into cells, presumably by adsorptive endocytosis. Furthermore, PEI is only partially protonated at physiological pH. Upon acidification within the endosome/ lysosome, PEI presumably acts as proton sponge with the protonation triggering chloride influx, osmotic swelling, and destabilization of the endosomal/ lysosomal vesicle enabling the release of the DNA into the cytosol. As a result, efficient gene transfer is obtained without the need for additional endosome destabilizing agents. The modified, PEI-conjugated, transferrin molecules maintain their ability to bind their cognate receptor and to mediate efficient iron transport into the cell. Transferrin receptor-mediated endocytosis occurs followed by the expression of the imported DNA. Deferrioxamine, a cell permeable iron chelator, increases the transferrin receptor density on the cell surface thus further enhancing gene delivery to the cell.

The concentration of Pei 40K, the main component of this product, is 1 mg / ml.

Storage and Handling Conditions

Wet ice bag transportation; Stored at 4℃ temperature, valid for 6 months. For longer periods, stored at -20℃， avoid repeated freeze-thaw cycles.

Component

Component Number	Component	G1802-1ML	G1802-10ML
G1802-1	PEI 40 K Transfection Reagent	1 mL	10 mL
Mamual		1 pc	1 pc

Assay Protocol / Procedures

1. Preparation before transfection of adherent cells (6-well plate, reference table for other specifications):
   A) The day before transfection, cells were appropriate to be uniformly planted in the pore plates at an appropriate density to converge to 70 – 85% upon formal transfection;
   B) Before transfection, primary cell media was removed and washed 1-twice with PBS before replacing 1.8 mL of base medium without serum or low serum (below 5%), or Opti-MEM; different cells had different serum dependence depending on the specific cell type;
   C) The apped cells were placed in the incubator and preparation of transfection complexes was started.
2. Suspension cells were prepared before transfection:
   A) On the day of transfection, an appropriate amount of cells were centrifuged to remove the medium and resuspended with basal medium without serum or low serum (below 5%);
   B) The prepared suspension cells were placed in the incubator and started the preparation of transfection complexes, and the volume ratio between transfection complexes and medium could be made with reference to adherent cells for appropriate adjustments.
3. Transfection complexes were prepared:
   A) Preparation liquid A: 100 μL of serum-free base medium and 2 μg of plasmid DNA, blowing and mixed;
   B) Preparation of liquid B: 100 L of base medium without serum and 6-8 μL PEI 40 K Transfection Reagent, blowing and mixing;
   C) Solution B was added to liquid A, gently blown and mixed, and incubated at room temperature for 15 min to form a DNA-PEI transfection complex.
4. Transfected cells:
   A) Add the transfection complex obtained in the previous step to the previously changed hole plate by crossing or surrounding the solution;
   B) Shake the plate "8" or other way to be fully mixed and incubated in a 37℃, 5% CO2 incubator;
   C) DNA-PEI transfection complexes with cells for 4 – 6 h showed better transfection efficiency, appropriate extension or overnight incubation and better transfection efficiency.

Note:

1. Please use healthy cells in good condition and recovered cells are recommended to transage at least 2 times before transfection.
2. Please use a high-quality, endotoxin-free, plasmid DNA.
3. High concentrations of serum at transfection, as well as the use of antibiotics, may affect cell status as well as transfection efficiency.
4. Different cell tolerance, sensitivity, length of transfection incubation time, and PEI / DNA usage ratio were adjusted as appropriate.
5. Please wear experimental suits and disposable gloves when operation.

Schedule: Use of transfection in different cell culture containers (for reference only)

Cultivate utensils	Growth area (cm^2)	Number of inoculated cells	DNA content (μg)	PEI (μL)	Transfection complex volume (μL)	Total volume (mL)
96-well plates	0.3	$(2-4)\times10^4$	0.1	0.3-0.4	10	0.1
24-well plates	1.9	$(1.2-2.4)\times10^5$	0.5-1	1.5-4	50	0.5
12-well plates	3.8	$(2.4-4.8)\times10^5$	1-2	3-8	100	1
6-well plates	9.5	$(6-10)\times10^5$	2-4	6-16	200	2
10 cm culture dish	55	$(4-6)\times10^6$	12-24	36-96	1000	12
T75 culture flask	75	$(6-10)\times10^6$	18-36	54-144	1000	15

Servicebio® PEI 40K Transfection Reagent

Cat. #：G1802

Product information

product name	Identification of product	model
PEI 40K Transfection Reagent	G1802-1ML	1 mL
	G1802-10ML	10 mL

Descirption/Introduction

PEI 40K Transfection Reagent (linearized polyethyleneimine transfection reagent) is a highly charged cationic polymer with a molecular weight of 40,000 based on linearized polyethyleneimine, which is positively charged and can effectively bind negatively charged nucleic acids to form a complex with them and introduce them into the cells, and it is suitable for the transfection of plasmid DNA in cells. PEI 40K (linearized polyethyleneimine) transfection reagent has low cytotoxicity, high transfection efficiency and low cost, making it very cost-effective compared to liposome-based transfection reagents. It has been validated that the linear PEI 40K transfection reagent is widely used in a variety of cell lines including HEK-293, HEK293T, CHO-K1, COS-1, COS-7, NIH/3T3, Sf9, HepG2, and Hela cells, etc., with transfection efficiencies as high as 80%~90%. The concentration of PEI 40K, the main component of this product, is 1 mg/mL.

Storage and Handling Conditions

Ship with wet ice; Store at -20℃, valid for 6 months. Or store at 4℃, valid for 3 months.

Component

Component Number	Component	G1802-1ML	G1802-10ML
G1802-1	PEI 40 K Transfection Reagent	1 mL	10 mL
Mamual		1 pc	1 pc

Assay Protocol / Procedures

1. Preparation before transfection of adherent cells (6-well plate, reference table for other specifications):
   A) The day before transfection, cells were appropriate to be uniformly planted in the pore plates at an appropriate density to converge to 70 – 85% upon formal transfection;
   B) Before transfection, primary cell media was removed and washed 1-2 times with PBS before replacing 1.8 mL of base medium without serum or low serum (below 5%), or Opti-MEM; different cells had different serum dependence depending on the specific cell type;
   C) The apped cells were placed in the incubator and preparation of transfection complexes was started.
2. Suspension cells were prepared before transfection:
   A) On the day of transfection, an appropriate amount of cells were centrifuged to remove the medium
   and resuspended with basal medium without serum or low serum (below 5%);
   B) The prepared suspension cells were placed in the incubator and started the preparation of transfection complexes, and the volume ratio between transfection complexes and medium could be made with reference to adherent cells for appropriate adjustments.
3. Transfection complexes were prepared:

A) Preparation liquid A: 100 μL of serum-free base medium and 2 μg of plasmid DNA, blowing and mixed;

B) Preparation of liquid B: 100 L of base medium without serum and 6-8 μL PEI 40 K Transfection Reagent, blowing and mixing;

C) Solution B was added to liquid A, gently blown and mixed, and incubated at room temperature for 15 min to form a DNA-PEI transfection complex.

4. Transfected cells:

A) Add the transfection complex obtained in the previous step to the previously changed hole plate by crossing or surrounding the solution;

B) Shake the plate "8" or other way to be fully mixed and incubated in a 37℃, 5% CO2 incubator;

C) DNA-PEI transfection complexes with cells for 4 – 6 h showed better transfection efficiency, appropriate extension or overnight incubation and better transfection efficiency.

Note:

1. Please use healthy cells in good condition and recovered cells are recommended to be passaged at least 2 times before transfection.
2. Please use a high-quality, endotoxin-free, plasmid DNA.
3. For initial use, it is recommended that the product be dispensed to avoid repeated freezing and thawing.
4. High concentrations of serum at transfection, as well as the use of antibiotics, may affect cell status as well as transfection efficiency.
5. Different cell tolerance, sensitivity, length of transfection incubation time, and PEI / DNA usage ratio were adjusted as appropriate.
6. Please wear experimental suits and disposable gloves when operation.

Schedule: Use of transfection in different cell culture containers (for reference only)

Cultivate utensils	Growth area (cm^2)	Number of inoculated cells	DNA content (μg)	PEI (μL)	Transfection complex volume (μL)	Total volume (mL)
96-well plates	0.3	$(2-4)\times10^4$	0.1	0.3-0.4	10	0.1
24-well plates	1.9	$(1.2-2.4)\times10^5$	0.5-1	1.5-4	50	0.5
12-well plates	3.8	$(2.4-4.8)\times10^5$	1-2	3-8	100	1
6-well plates	9.5	$(6-10)\times10^5$	2-4	6-16	200	2
10 cm culture dish	55	$(4-6)\times10^6$	12-24	36-96	1000	12
T75 culture flask	75	$(6-10)\times10^6$	18-36	54-144	1000	15

Servicebio® Polybrene (10 mg/mL)

Cat. #：G1803-1ML

Product Information

Product Name	Cat. No	Spec.
Polybrene (10 mg/mL)	G1803-1ML	1 mL

Product Description/Introduction

Polybrene, also known as Hexadimethrine Bromide, is a class of polycationic polymers that are positively charged when dissolved and can bind to cell surface anions, and is commonly used in virus-mediated infections of mammalian cells by lentiviruses, adenoviruses and other viruses, and can significantly increase the efficiency of lentiviral and adenoviral infections of certain cells. The mechanism of action may be to promote adsorption by neutralizing the electrostatic repulsion between cell surface salivary acids and virus particles. Polybrene is also a well-known anti-heparin agent (heparin antagonist) and is commonly used to produce non-specifically agglutinated red blood cells. In addition, polybrene is also used in protein sequencing, because small doses of polybrene can significantly improve the degradation of peptides in automated sequencing analysis. The addition of polybrene to PVDF membranes also improves the affinity of the membrane. The concentration of the main component of this product is 10 mg/mL, and it is filtered by microporous membrane to remove bacteria.

Storage and Shipping Conditions

Ship with wet ice; stored at 4℃, valid for 12 months; stored at -20℃, valid for 24 months. Avoid repeated freezing and thawing

Product Contents

Component	G1803
Polybrene (10 mg/mL)	1 mL
Manual	One copy

Assay Protocol / Procedures

1. According to the experimental requirements, Plant cells in the well plate and incubate plate overnight.
2. According to the MOI value obtained from relevant literature or pre-experiment, dilute the virus stock solution with complete medium to the desired concentration. And then add appropriate amount of Polybrene (10 mg/mL) to a final concentration of 2-12μg/ mL. (5-8 μg/mL is generally used).
3. Discard cell culture medium in the well plate. The virus solution-medium-polybrene mixture was added to the well plates, shaken gently and incubated in an incubator;
4. Incubate for 6-16 h in a cell culture incubator. Discard the culture medium containing virus, replace it with fresh complete medium (at this time, appropriate antibiotics may be added for screening), and continue to incubate (the incubation time of virus depends on the specific situation, if the cell is in good condition, the incubation time can be appropriately extended; Otherwise, it needs to be replaced with normal medium in advance).
5. According to the purpose of the experiment, the cells are collected or detected after a certain period of incubation.

Note

1. Different cells have different sensitivities to Polybrene, so it is recommended to do cytotoxicity test for the first experiment.
2. If stored at -20℃, it is recommended that the storage be properly packed to avoid freeze - thaw cycles.
3. For your safty and health, please wear safety glasses, gloves, or protective clothing.

Servicebio® SweTransDNA Transfection Reagent

Cat. No.: G1805-0.5ML

Product Information

Product Name	Cat. No.	Spec.
SweTransDNA Transfection Reagent	G1805-0.5ML	0.5 mL

Product Description/Introduction

This product is a new generation of DNA transfection reagent with excellent performance of cationic polymer type, which has superb DNA binding and delivery ability, and can be used for transfection of plasmid DNA. This product has the advantages of very low toxicity, good stability, simple and easy transfection, good reproducibility, etc. It can be used for transient transfection and stable transfection of many types of cells.

Storage and Shipping Conditions

Ship with wet ice; Store at 2-8℃ for 12 months.

Product Content

Component	G1805-0.5ML
SweTransDNA Transfection Reagent	0.5 mL
Manual	1 pc

Assay Protocol / Procedures

1. **Pre-transfection preparation of adherent cells (6-well plate for example, other specifications refer to the attached table):**
   a. One day before transfection, the cells are planted uniformly in the well plate at a suitable density so that the cells converge to 60-85% during formal transfection;
   b. Before transfection, remove the original cell culture medium, wash the cells with room temperature PBS buffer for 1-2 times, and then replace with 1.9 mL of serum-free or low-serum (less than 5%) basal medium (different cells have different serum dependency, please adjust the serum percentage as appropriate);
   c. Cells are placed in a cell culture incubator after fluid exchange and transfection complexes are prepared;
2. **Preparation of suspension cells for transfection:**
   a. On the day of transfection, after centrifuging the appropriate amount of cells to remove the medium, resuspend the cells in serum-free or low-serum (5% or less) basal medium to the appropriate concentration, and inoculate 1.9 mL of the resuspension uniformly in the well plate;
   b. Place the well plate inoculated with suspended cells in a cell culture incubator and start to prepare the transfection complex (the volume ratio between the transfection complex and the culture medium can be adjusted appropriately by referring to the ratio of adherent cells);
3. **Transfection complex preparation:**
   a. Prepare Solution A: 50 μL of serum-free basal medium with 2 μg of plasmid DNA, blown up and mixed well;
   b. Preparation of solution B: 50 μL of serum-free basal medium with 2-10 μL of SweTransDNA transfection reagent was blown and mixed;

c. Leave Liquid A and Liquid B at room temperature for 5 minutes;

d. Add liquid B to liquid A, gently blow to mix well, and continue to incubate at room temperature for 20 min to form the DNA-transfection reagent complex;

4. Transfection of cells:

a. The DNA-transfection reagent complex obtained in the previous step is added to the orifice plate with the liquid replaced in "Step 1" or "step 2";

b. After gently shaking the culture plate to induce the medium containing the transfection complex to mix well with the medium in the well plate, the well plate is incubated at 37°C in a 5% CO_2 incubator;

c. After 4-6 h of incubation, the different cells are changed into the corresponding fresh medium and placed in the incubator for further incubation;

d. After 24-48 h of transfection, cells are observed or collected.

Note

1. Please use healthy cells in good condition, and resuscitated cells are recommended to be passaged at least twice before transfection.
2. Please use high quality, endotoxin-free plasmid DNA.
3. The product is used for the first time, it is recommended to pack separately to avoid repeated freezing and thawing.
4. Under the premise of cell tolerance, the incubation time of DNA-transfection reagent complex can be extended appropriately, and the transfection effect is better. The ratio of DNA to transfection reagents can be adjusted as appropriate.
5. For your health and safety, please wear lab coat and gloves during operation.

Ver. No.: V1.0-202308

Table: The amount of transfection reagents and DNA required for different culture plates

Culture dish	Single well area	Number of inoculated cells	DNA transfection		Total volume
			Transfection reagent	DNA	
96-well plate	0.3 cm²	(2.0-4.0)×10^4	0.2-1.0 μL	0.2 μg	0.1 mL
24-well plate	2.0 cm²	(1.2-2.4)×10^5	0.4-2.0 μL	0.4 μg	0.5 mL
12-well plate	4.0 cm²	(2.4-4.8)×10^5	1.0-5.0 μL	1.0 μg	1.0 mL
6-well plate	9.5 cm²	(6.0-10)×10^5	2.0-10 μL	2.0 μg	2.0 mL

Servicebio® SweTransRNA Transfection Reagent

Cat. No.: G1806-0.5ML

Product Information

Product Name	Cat. No.	Spec.
SweTransRNA Transfection Reagent	G1806-0.5ML	0.5 mL

Product Description/Introduction

This product is a high-performance cationic polymer-based RNA transfection reagent that delivers RNA directly to the cytoplasm of target cells without being degraded by nucleases. The gene expression process takes place in the cytoplasm without entering the nucleus and is not restricted by the transcriptional regulation of the target cell. This product has the advantages of little influence by serum, extremely low toxicity, good stability, easy operation and good repeatability. It can be applied to RNA transfection of various adherent cells and suspension cells.

Storage and Shipping Conditions

Ship with wet ice; Store at 2-8℃ for 12 months.

Product Content

Component	G1806-0.5ML
SweTransRNA Transfection Reagent	0.5 mL
Manual	1 pc

Assay Protocol / Procedures

1. **Pre-transfection preparation of adherent cells (6-well plate for example, other specifications refer to the attached table):**
   a. One day before transfection, the cells are planted uniformly in the well plate at a suitable density so that the cells converge to 70-85% during formal transfection;
   b. Before transfection, remove the original cell culture medium, wash the cells with room temperature PBS buffer for 1-2 times, and then replace with 1.9 mL of serum-free or low-serum (less than 5%) basal medium (different cells have different serum dependency, please adjust the serum percentage as appropriate);
   c. Cells are placed in a cell culture incubator after fluid exchange and transfection complexes are prepared;
2. **Preparation of suspension cells for transfection:**
   a. On the day of transfection, after centrifuging the appropriate amount of cells to remove the medium, resuspend the cells in serum-free or low-serum (5% or less) basal medium to the appropriate concentration, and inoculate 1.9 mL of the resuspension uniformly in the well plate;
   b. Place the well plate inoculated with suspended cells in a cell culture incubator and start to prepare the transfection complex (the volume ratio between the transfection complex and the culture medium can be adjusted appropriately by referring to the ratio of adherent cells);
3. **Transfection complex preparation:**
   a. Prepare Solution A: 50 μL of serum-free basal medium with 80 pmol of RNA, blown up and mixed;
   b. Preparation of solution B: 50 μL of serum-free basal medium with 4-20 μL of SweTransRNA

transfection reagent, blown up and mixed;

c. Leave Liquid A and Liquid B at room temperature for 5 minutes;

d. Add solution B to solution A, gently blow to mix well, and continue to incubate at room temperature for 15-20 min to form the RNA-transfection reagent complex;

4. Transfection of cells:

a. The RNA-transfection reagent complex obtained in the previous step is added to the orifice plate with the liquid replaced in "Step 1" or "step 2";

b. After gently shaking the culture plate to induce the medium containing the transfection complex to mix well with the medium in the well plate, the well plate is incubated at 37°C in a 5% CO_2 incubator;

c. After 4-6 h of incubation, the different cells are changed into the corresponding fresh medium and placed in the incubator for further incubation;

d. After 24-48 h of transfection, cells are observed or collected.

Note

1. Please use healthy cells in good condition, and resuscitated cells are recommended to be passaged at least twice before transfection.
2. Please use high quality RNA for transfection.
3. The product is used for the first time, it is recommended to pack separately to avoid repeated freezing and thawing.
4. Under the premise of cell tolerance, the incubation time of RNA-transfection reagent complex can be extended appropriately, and the transfection effect is better. The ratio of RNA to transfection reagents can be adjusted as appropriate.
5. For your health and safety, please wear lab coat and gloves during operation.

Table: The amount of transfection reagents and RNA required for different culture plates

Culture dish	Single well area	Number of inoculated cells	RNA transfection		Total volume
			Transfection reagent	RNA	
96-well plate	0.3 cm^2	$(2.0-4.0)\times10^4$	0.25-1.25 μL	5 pmol	0.1 mL
24-well plate	2.0 cm^2	$(1.2-2.4)\times10^5$	1.0-5.0 μL	20 pmol	0.5 mL
12-well plate	4.0 cm^2	$(2.4-4.8)\times10^5$	2.0-10 μL	40 pmol	1.0 mL
6-well plate	9.5 cm^2	$(6.0-10)\times10^5$	4.0-20 μL	80 pmol	2.0 mL

Servicebio® Chromatographic Column for Separating Exosomes (Serum/Plasma)

Cat. No.: G4112

Product Information

Product Name	Cat. No.	Spec.
Chromatographic Column for Separating Exosomes (Serum/Plasma)	G4112-5	5

Product Description/Introduction

Exosomes exist naturally in body fluids such as blood, saliva, urine, and breast milk. They are nanoscale membranous vesicles with uniform size and particle size of 30 to 150 nm that are actively secreted by cells into the extracellular space. This vesicle has its own specific markers, and is coated with proteins, mRNA, miRNA, lipids, and other substances, which can be used as a pathway for substance and signal communication between cells and participate in processes of cell growth, cell proliferation, cell differentiation, cell migration, angiogenesis, and tumor growth.

This product is a size exclusion chromatography column (SEC) suitable for rapid separation and purification of serum/plasma exosomes. It can retain high-purity and highly naturally active exosomes while efficiently removing proteins; Effectively avoiding the damage to the extracellular membrane structure caused by ultra-high centrifugal force in traditional ultracentrifugation methods, while reducing the impact of high concentration proteins and other substances on the purity of exosomes in traditional precipitation methods.

Storage and Shipping Conditions

Transportation at room temperature; Store at room temperature with a validity period of 12 months.

Product Content

Component	G4112-5
Chromatographic Column for Separating Exosomes (Serum/Plasma)	5 pieces
Product Manual	1 pc

Assay Protocol / Procedures

1. **Preparation**
   a) Prepare a room temperature, sterile buffer solution, such as PBS (recommended G4202);
   b) Prepare room temperature, sterile 0.1M NaOH reagent and 20% ethanol;
   c) Before using the separation column, remove the red upper cover of the separation column and place it on a bracket (recommended G6063) for use;
2. **Exosomes separation**
   a) Using serum/plasma biological samples 0.22 μm membrane filtration, preliminary purification for backup;
   b) Remove the white bottom cover of the separation column, and after the protective liquid inside the column flows out, add a total of 30 mL buffer solution liquid in batches to clean the separation column;
   c) After all the buffer above the sieve plate enters the separation column; Take 0.5mL of the backup serum/plasma from step **2.a)** and add it to the center of the sieve plate (if less than 0.5mL, add buffer solution to make up to 0.5mL);

d) After all serum/plasma passes through the sieve plate and enters the separation column, add 2.5mL buffer solution;

e) After all the buffer solution in the previous step passes through the sieve plate and enters the separation column, add 1 mL of buffer solution. At the same time, collect 1 mL of washout solution, which is rich in naturally active exosomes and can be used for subsequent experiments or frozen at -80 ℃ for backup;

3. Post separation treatment

a) After completing the separation of exosomes, add a total of 30 mL of buffer solution in batches to clean the separation column;

b) If it is necessary to continue using the separation column to separate serum/plasma exosomes, repeat steps **2.c)** to **2.e)**;

c) If it is not necessary to continue using the separation column to separate serum/plasma exosomes, first add 5 mL of 0.1M NaOH to clean the separation column, then add a total of 20 mL of buffer solution in batches to clean and balance the separation column, and then add 5 mL of 20% ethanol to clean the separation column. After cleaning, cover the white bottom cover of the separation column; Finally, add 2mL of 20% ethanol to moisturize, cover and tighten the red top of the separation column, and store the separation column at room temperature.

Note

1. When separating exosomes from different serum/plasma, the elution curve and purity of this product may slightly differ, but it does not affect the overall trend.
2. When the sample size is large or the content of exosomes in the sample is low, the sample can be concentrated first and then separated using a separation chromatography column.
3. During the process of separating serum/plasma exosomes from this product, it is necessary to avoid prolonged interruption of liquid flow in the separation column.
4. During the use of this product, do not separate biological samples from different sources on the same column, and do not reuse the same column more than 5 times.
5. This product is suitable for storage and use at room temperature, and low temperature use and high temperature heating should be avoided.
6. During the experiment, please wear lab clothes and disposable gloves to avoid contamination and ensure safety.

Servicebio® Chromatographic Column for Separating Exosomes （Cell Supernatant）

Cat. No.：G4113

Product Information

Product Name	Cat. No.	Spec.
Chromatographic Column for Separating Exosomes （Cell Supernatant）	G4113-5	5 pieces

Product Description/Introduction

Exosomes are nanoscale membranous vesicles with a uniform size and a particle size of 30 - 150 nm that are actively secreted into the extracellular space by cells. This vesicles have their own specific markers, and are coated with proteins, mRNA, miRNA, lipids, and other substances, which can be used as a pathway for substance and signal communication between cells and participate in processes such as cell growth, cell proliferation, cell differentiation, cell migration, angiogenesis, and tumor growth.

This product is a size exclusion chromatography column (SEC) suitable for rapid separation and purification of exosomes from cell supernatants. This product uses a cell supernatant concentration agent to separate and enrich cell supernatant exosomes, and then uses an extracellular vesicle separation chromatography column to purify the concentrated exosomes. Which can not only retain high-purity and high activity exosomes, but also efficiently remove proteins; Effectively avoiding the damage to the extracellular membrane structure caused by ultra-high centrifugal force in the ultra-high speed centrifugation method, and reduce the influence of high concentration proteins and other substances on the purity of exosomes in traditional precipitation methods.

Storage and Shipping Conditions

Transportation at room temperature; Store at room temperature with a validity period of 12 months.

Product Content

Component Number	Component	G4113-5
G4113-1	Chromatographic Column for Separating Exosomes （Cell Supernatant）	5 pieces
G4113-2	Cell supernatant concentrate	250mL
Product Manual		1 pc

Assay Protocol / Procedures

1. **Cell supernatant concentration**
   a) The biological samples of cell supernatant are filtered by 0.22 μM filter membrane and preliminary purified for use;
   b) Mix the cell supernatant biological sample and cell supernatant concentrate in equal volume, shake vigorously for 15 seconds, and let it stand for 2 hours in a refrigerator at 4 ℃;
   c) Place the mixture in a centrifuge at 4 ℃ at 10000 × g for 60 minutes; Discard the supernatant;
   d) 1 × PBS buffer of 5% volume of cell supernatant biological sample is added to fully resuspension centrifuge precipitation;
   e) The heavy suspension is rich in exosomes, which can be used for subsequent experiments or frozen at -80 ℃ for backup.
2. **Preparation for purification of exosomes**

a) Prepare a room temperature, sterile buffer solution, such as PBS (recommended G4202);
b) prepare room temperature, sterile 0.1M NaOH reagent and 20% ethanol;
c) Before using the separation column, remove the red upper cover of the separation column and place it on a bracket (recommended G6063) for use;

3. **Exosomes separation**
   a) Prepare exosome samples after concentration and resuspension of cell supernatant concentrate for later use;
   b) Remove the white bottom cover of the separation column, and after the protective liquid inside the column flows out, add a total of 30 mL of buffer solution in batches to clean the separation column;
   c) After all the buffer above the sieve plate enters the separation column; Take 0.5mL of the spare exosome sample from step **3.a)** and add it to the center of the sieve plate (if less than 0.5mL, add buffer solution to make up to 0.5mL);
   d) After all exosome samples pass through the sieve plate and enter the separation column, add 2.5mL buffer solution;
   e) After all the buffer solution in the previous step passes through the sieve plate and enters the separation column, add 1mL of buffer solution, and at the same time collect 1mL of washout solution, which is rich in naturally active exosomes; It can be applied for subsequent experiments or frozen at -80 ℃ for backup;
4. **Post separation treatment**
   a) After completing the separation of exosomes, add a total of 30 mL of buffer solution in batches to clean the separation column;
   b) If it is necessary to continue using the separation column to separate the exosomes from the cell supernatant, repeat steps **3.c)** to **3.e)**;
   c) If it is not necessary to continue using the separation column to separate exosomes from the cell supernatant, first add 5 mL of 0.1M NaOH to clean the separation column, then add a total of 20 mL of buffer solution in batches to clean and balance the separation column, and then add 5 mL of 20% ethanol to clean the separation column. After cleaning, cover the white bottom cover of the separation column; Finally, add 2mL of 20% ethanol to moisturize, cover and tighten the red top of the separation column, and store the separation column at room temperature.

Note

1. When separating exosomes from different cell supernatants using this product, the elution curve and purity slightly differ, but it does not affect the overall trend.
2. During the process of separating cell supernatant exosomes from this product, it is necessary to avoid prolonged interruption of liquid flow in the separation column.
3. During the use of this product, do not separate biological samples from different sources on the same column, and do not reuse the same column more than 5 times.
4. This product is suitable for storage and use at room temperature, and low temperature use and high temperature heating should be avoided.
5. During the experiment, please wear lab clothes and disposable gloves to avoid contamination and ensure safety.

Servicebio® Exosome Concentration Solution

Cat. No.: G4114

Product Information

Product Name	Cat. No.	Spec.
Exosome Concentration Solution	G4114-250ML	250 mL

Product Description/Introduction

Exosomes are nanoscale membranous vesicles of uniform size, with a particle size ranging from 30 to 150 nm, actively secreted by cells into the extracellular space. This vesicle has its own specific biomarker, and contains proteins, mRNA, miRNA, lipids and other substances, which can participate in the process of cell growth, proliferation, differentiation, migration, angiogenesis and tumor growth through inter-cell material and signal communication.

This product is a common concentrated solution of Exosomes, which can quickly obtain a large number of complete Exosomes particles from liquid biological samples such as cell supernatant and animal serum.

Storage and Shipping Conditions

Transportation at room temperature; Store at room temperature with a validity period of 12 months.

Product Content

Component	G4112-250ML
Exosome Concentration Solution	250 mL
Product Manual	1 pc

Assay Protocol / Procedures

1. **Sample pretreatment:**
   a) **Low content exosome biological samples** (using "**Cell Supernatant**" as an example): Take the cell supernatant, centrifuge at 4℃, 3000×g for 10 minutes to remove cell debris; Centrifuge the supernatant again at 4℃ and 10000×g for 20 minutes.Then filter the supernatant using a 0.22 μm filter membrane and purify it for later use.
   b) **High content exosome biological samples** (using "**Animal Serum**" as an example): Take animal serum, centrifuge at 4℃, 3000×g for 10 minutes fist; Then Centrifuge the supernatant at 4℃ and 10000 × g for 20 minutes.Last filter the supernatant using a 0.22 μm filter membrane. After preliminary purification, dilute the animal serum in a ratio of 1:9 to 1×PBS volume for later use.
2. **Exosome concentration:**
   a) Take the processed biological sample and mix it with an equal volume of exosome concentrate. After intense vortex oscillation for 15 s-60 s, let it stand for 2 hours at 4 ℃ in a refrigerator (Optional: Vortex oscillation every 30 minutes during the stationary period).
   b) Place the mixed solution in a centrifuge, centrifuge at 4 ℃ and 10000×g for 60 minutes; Discard the supernatant.
   c) Add pre-cooled PBS buffer in a ratio of 1 x PBS volume=100:1 according to the volume of exosome concentrate, and resuspend and centrifuge for precipitation (note: this is equivalent to 100×concentration, and different volumes of PBS can be added as needed for resuspension).
   d) Transfer the heavy suspension to a 1.5mL centrifuge tube and place it in a centrifuge. Centrifuge at 4 ℃ and 12000 rpm for 5 minutes; Collect supernatant.
   e) The supernatant is rich in exosomes and can be used for subsequent experiments. It can also be packaged and stored at -80 ℃ for future use.

Note

1. This product can be stored stably at room temperature for a long time. Please shake and mix well before use.
2. This product can be used as a supplement to the cell supernatant concentration in the G4113 exosome separation chromatography column (cell supernatant).
3. During the experiment, please wear lab clothes and disposable gloves to avoid contamination and ensure safety.

По вопросам продаж и поддержки обращайтесь:

Алматы (7273)495-231
Ангарск (3955)60-70-56
Архангельск (8182)63-90-72
Астрахань (8512)99-46-04
Барнаул (3852)73-04-60
Белгород (4722)40-23-64
Благовещенск (4162)22-76-07
Брянск (4832)59-03-52
Владивосток (423)249-28-31
Владикавказ (8672)28-90-48
Владимир (4922)49-43-18
Волгоград (844)278-03-48
Вологда (8172)26-41-59
Воронеж (473)204-51-73
Екатеринбург (343)384-55-89
Иваново (4932)77-34-06
Ижевск (3412)26-03-58
Иркутск (395)279-98-46
Казань (843)206-01-48
Калининград (4012)72-03-81
Калуга (4842)92-23-67
Кемерово (3842)65-04-62
Киров (8332)68-02-04
Коломна (4966)23-41-49
Кострома (4942)77-07-48
Краснодар (861)203-40-90
Красноярск (391)204-63-61
Курск (4712)77-13-04
Курган (3522)50-90-47
Липецк (4742)52-20-81
Магнитогорск (3519)55-03-13
Москва (495)268-04-70
Мурманск (8152)59-64-93
Набережные Челны (8552)20-53-41
Нижний Новгород (831)429-08-12
Новокузнецк (3843)20-46-81
Ноябрьск (3496)41-32-12
Новосибирск (383)227-86-73
Омск (3812)21-46-40
Орел (4862)44-53-42
Оренбург (3532)37-68-04
Пенза (8412)22-31-16
Петрозаводск (8142)55-98-37
Псков (8112)59-10-37
Пермь (342)205-81-47
Ростов-на-Дону (863)308-18-15
Рязань (4912)46-61-64
Самара (846)206-03-16
Санкт-Петербург (812)309-46-40
Саратов (845)249-38-78
Севастополь (8692)22-31-93
Саранск (8342)22-96-24
Симферополь (3652)67-13-56
Смоленск (4812)29-41-54
Сочи (862)225-72-31
Ставрополь (8652)20-65-13
Сургут (3462)77-98-35
Сыктывкар (8212)25-95-17
Тамбов (4752)50-40-97
Тверь (4822)63-31-35
Тольятти (8482)63-91-07
Томск (3822)98-41-53
Тула (4872)33-79-87
Тюмень (3452)66-21-18
Ульяновск (8422)24-23-59
Улан-Удэ (3012)59-97-51
Уфа (347)229-48-12
Хабаровск (4212)92-98-04
Чебоксары (8352)28-53-07
Челябинск (351)202-03-61
Череповец (8202)49-02-64
Чита (3022)38-34-83
Якутск (4112)23-90-97
Ярославль (4852)69-52-93

Россия +7(495)268-04-70 Казахстан +7(7172)727-132 Киргизия +996(312)96-26-47

эл.почта: sih@nt-rt.ru || сайт: https://servicebio.nt-rt.ru/