Растворы для морфологического окрашивания, специальные пятна, ч. 2

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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:

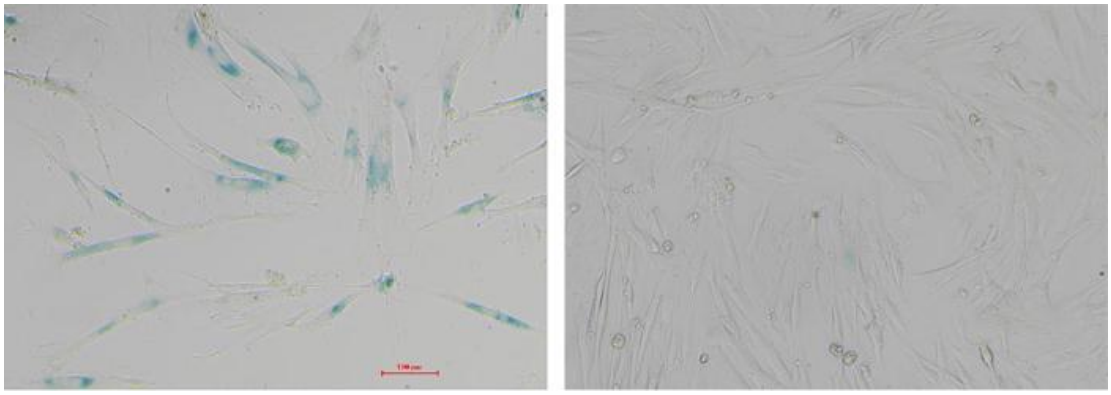


Fig.1 WI-38 cells were stained with β-galactosidase kit. The left picture shows senescence WI-38 cells without division and proliferation ability but still alive. After staining, the positive staining cells were more than 95%. The image on the right shows newly resuscitated WI-38 cells (early passage) with less than 3 passages, and no obvious positive cells after staining.

Servicebio® Magenta (For Marking)

Cat No.: G1061-250ML

Product Information

Product Name	Cat.No.	Spec.
Magenta (For Marking)	G1061-250ML	250 mL

Description

Magenta is a common biological stain. This product is labeled magenta. It is used for labeling laboratory animals or small tissue blocks. It is convenient to group experimental animals or identify small tissue blocks after labeling.

The active ingredient of this product is 0.8% basic fuchsin and the solvent is 70% ethanol.

Storage and Handling Conditions

Store and transport at room temperature; valid for 12 months.

Assay Protocol

1. The fur of experimental animals can be stained and marked according to the needs.

2. When the collected tissue is small and difficult to distinguish, the dye solution can be used for dyeing and then dehydrated embedding.

Note

Please wear a lab coat and disposable gloves during operation.

Servicebio® Von Kossa Staining Solution

Cat. No.: G1043-20ML

Product Information

Product Name	Cat.No.	Spec.
Von Kossa Staining Solution	G1043-20ML	3×20 mL

Description

This product is Von Kossa dye solution for calcium salt staining in tissues. Von Kossa staining is a classic method of staining mineralized nodules. The basic principle is that silver nitrate and insoluble calcium salts form reducible silver salts in situ through metathesis. Then under the action of strong light or ultraviolet light or strong reducing agent, the silver salt is reduced to black elemental silver to realize the color development of calcium salt in the tissue. It is suitable for the samples with more calcium salt deposition.

This product is a set containing cell nucleus and cytoplasm counterstaining solution, in which Von Kossa staining solution is XSY solution. After staining, the calcium salt deposit area is black, the nucleus is blue, and the background is light red.

Storage and Handling Conditions

Store and transport at room temperature; valid for 12 months.

Component

Component Number	Component	G1043-20ML
G1043-1	Von Kossa Solution	20 mL
G1043-2	Hematoxylin Solution	20 mL
G1043-3	Eosin Solution	20 mL
Product Manual		

Assay Protocol

1. Paraffin sections were successively dewaxed by xylene for 10 min, replaced by fresh xylene for 10 min, absolute ethanol for 5min, fresh absolute ethanol for 5min, 90% ethanol for 5min, 75% ethanol for 5min, and then immersed and washed with ultra-pure water for 5 times.

2. The tissue was circled with a tissue chemical pen, and the sections were placed in a transparent wet box, and Von Kossa dye solution was added to the sections to cover the tissue, capped, and the wet box was placed smoothly under ultraviolet light for 4 h. Note: If it is a high power UV lamp, irradiation time can be

shortened accordingly. Here 4 h irradiation with ordinary ultra-clean table UV lamp.

3. Wash Von Kossa dye with ultrapure water (must be rinsed), remove sections immediately, soak in ultrapure water for 3 times, and rinse with running water for 2 min.

4. Restaining: Sections were stained with hematoxylin dye solution for 3-5 min, washed with tap water, differentiated solution (**recommended G1039**) differentiated, washed with tap water, bluing solution (**recommended G1040**) returned to blue, washed with running water, and dehydrated with 85% and 95% alcohol gradient for 5 min each. The sections were stained with eosin solution for 5 min and dehydrated with absolute ethanol twice for 5 min each. Then the sections were dehydrated with fresh absolute ethanol for 5 min, transparent with xylene for 5 min, and transparent with fresh xylene for 5 min. Add drops of neutral gum to seal the slices.

Note: Prepare differentiation solution, bluing solution, xylene, gradient ethanol, neutral gum, etc.

Note

1. Before and after Von Kossa staining droplets, the sections were immersed in ultrapure water to avoid ionic impurities in the water interfering with the staining results.
2. If there is no ultraviolet lamp, you can also use strong sunlight irradiation, pay attention to adjust the irradiation time.
3. Each set of staining solution can be used to stain approximately 20 sections. One of the Von Kossa stains is not reusable and requires drop staining. Hematoxylin and eosin stains are dip-stained and can be reused.
4. This product is not suitable for decalcified tissues and tissues with less calcium salt deposition. Alizarin Red S staining (**G1038**) is recommended for tissues with low calcium salt deposition.

Servicebio® Toluidine Blue Dye Solution

Cat No.: G1032-100ML

Product Information

Product Name	Cat.No.	Spec.
Toluidine Blue Dye Solution	G1032-100ML	100 mL

Description

Toluidine Blue is a quinone imine basic staining, which can combine with acidic substances in tissue cells to achieve tissue staining. The nuclei and Nissl's body in neurons is stained into blue with toluidine blue, which can be used for the preliminary pathological diagnosis of Nissl body; The cytoplasm of mast cells contains heparin and histamine, and the cartilage contains chondroitin sulfate. These substances have metachromatic properties and are stained into purplish red with toluidine blue. Therefore, toluidine blue staining can be used to observe the distribution and abnormal changes of mast cells and the morphological structure of cartilage, such as tide lines; plant tissues can be stained and observed, the xylem, ducts, sieve tubes and other structures, the xylem are stained into blue-green and the cellulose cell wall is stained into blue-purple.

The active ingredient concentration of the toluidine blue dye solution of this product is 0.5%, which can be used for the staining of conventional animal and plant tissue sections.

Storage and Handling Conditions

Transport at room temperature; Valid for 18 months.

Component

Component	G1032
Toluidine Blue Dye Solution	100 mL
Manual	1 pc

Assay Protocol / Procedures

1. Paraffin sections were dewaxed to water;
2. Toluidine blue stain: Put the tissue sections into the toluidine blue dye solution for 2-5 minutes, wash slightly with tap water to remove excess dye.
   a. For plant tissue: Controll the degree of staining under the microscope,and according to the degree of staining, use 0.1% glacial acetic acid for proper differentiation. If the degree of coloring is appropriate and does not require differentiation, the slices are dried in a 60°C oven.
   b. For animal tissue: Wash animal tissue sections with water and differentiated with 0.1% glacial acetic acid. The degree of differentiation was observed microscopically, and differentiation was sufficient until the background was light blue and the coloration of the nidus, cartilage, and mast cells was evident. Rinse with tap water to terminate differentiation and then dry it in an oven at 60℃.

3. Transparent and mount: Sections were transparent to xylene for 10 min and then sealed with neutral gum.

Note

1. The washing time of the stained sections should not be too long, otherwise the color will fade easily.
2. Sections must be completely dried prior to transparent sealing so that any remaining tiny droplets of water do not interfere with observation.
3. Staining Solution is reusable. 100 mL of Staining Solution can be used to stain (dip or drop) approximately 350 sections. Replace the stain with a new one when the tissue or cell coloring is significantly lighter or abnormal.
4. Please wear lab coat and disposable gloves during operation.

Servicebio® Oil Red O solution

Cat No.: G1015-100ML

Product Information

Product Name	Cat.No.	Spec.
Oil Red O solution	G1015-100ML	100 mL

Description

Oil red O, also known as Sudan red 5B, is a fat soluble azo dye. This dye can specifically stain neutral lipids such as triglycerides in cells or tissues, but it is weak for phospholipids and steroids. The basic principle is that oil red O dissolves in lipids to make lipids red to orange red.

The saturated oil red O dye solution of this product is the saturated solution of oil red O. It can be used to dye tissue sections or cells after being diluted with distilled water before use. After dyeing, the fat drops in the tissues are red to orange red.

Storage and Handling Conditions

Storage and transportation under room temperature and away from light, valid for 12 months

Component

Component	G1015-100ML
Oil Red O solution	100 mL
Product Manual	

Assay Protocol

Preparation of working solution: Before use, 6 parts of saturated oil red O dye solution are thoroughly mixed with 4 parts of distilled water, and left to stand overnight at 4 ℃. The next day, filter once with qualitative filter paper, and then filter again at 4 ℃ for 24 hours to obtain the oil red O working solution. In addition, 60% isopropanol is prepared.

I Sample preparation

1. For cells: aspirate the cell culture medium, slowly add PBS (G4202 is recommended) to the edge of the orifice plate to simply clean the cells. Add 4% paraformaldehyde fixative (G1101 is recommended) and fix it at room temperature for 8-10 min, and rinse it twice with PBS.

2. For frozen sections: take out the sections from - 20 ℃ and let them stand at room temperature for 5-10 min to recover to normal temperature.

I Dyeing steps

1. For cells

(1) Add a small amount of 60% isopropyl alcohol into the pore plate to cover the cells for 15-20 seconds, and then suck out 60% isopropyl alcohol and dry the water slightly.

(2) Add oil red O working solution to the orifice plate to cover the cells, and dye them at room temperature in dark for 30 min to remove the dye.

(3) Add 60% isopropanol for rapid differentiation for 3-5 seconds, wash with pure water for 3 times, and each

time for 5 minutes.

(4) (Optional) Add hematoxylin dye solution (**G1004 is recommended**) to dye the nucleus, wash with water, turn blue and then wash with water.

(5) PBS was added to cover the cells and observed under microscope. In case of cell climbing, glycerol gelatin film sealant (**G1402 is recommended**) can be used for slide mounting.

2. For frozen sections

(1) Frozen sections recovered to room temperature were gently immersed in oil Red O working solution and stained for 8-10 min (covered to avoid light).

(2) The sections were taken out, stayed for 3 s, and then immersed in two cylinders of 60% isopropanol for differentiation for 3-5 s.

(3) Sections were immersed in two cylinders of pure water for 10 s each time.

(4) (Optional) The sections were immersed in hematoxylin dye solution to stain the nuclei, washed with water, then returned to blue and washed again. After slightly drying, glycerin gelatin was added to mount the slide.

Note:

1. If it is a frozen section of fresh tissue, the section should be fixed before staining.
2. During the whole operation, pay attention to the gentle action to avoid fat loss or displacement.
3. Samples stained with oil Red O cannot be stored for a long time, and should be observed and photographed as soon as possible.
4. When using glycerin gelatin to mount slides, attention should be paid to avoid bubbles as far as possible. If bubbles are not allowed to press the glass slide or forcibly tear the cover glass after mounting the slides, it will cause fat displacement. The slide can be immersed in warm water at 50-60 °C to allow the cover slide to fall off and then re-mount the slide.
5. This product can be used to stain approximately 80 sections. Replace with new stain when tissue or cell staining is significantly light or abnormal in color.
6. For your safety and health, please wear a lab coat and disposable gloves during operation.

Servicebio® PAS Staining Kit

Cat No.: G1008

Product Information

Product Name	Cat.No.	Spec.
PAS Staining Kit	G1008-20ML	3×20 ml
	G1008-100ML	3×100 ml

Product Introduction

This product PAS stain is used for glycogen PAS stain (Periodic Acid-Schiff stain), which is mainly used in tissue morphology to detect glycogen and polysaccharides in tissues. The main principle is to oxidize the hydroxyl groups on the two adjacent carbon atoms of sugar substances to aldehyde groups by the oxidizing agent periodic acid, and then react with the aldehyde groups to produce a purplish-red substance by using Schiff's reagent.

This product is a set, in which Solution A is Schiff Reagent, Solution B is 0.5% periodate solution, and Solution C is hematoxylin stain. After staining with PAS staining solution of this product, glycogen, neutral mucus material, cartilage matrix, epithelial basement membrane, plant fungi and cell wall in the tissues are purplish-red, and the nuclei of the cells are light blue.

Storage and Handling Conditions

The whole set of reagents can be stored at 2-8℃ away from light, of which PAS Solution B and PAS Solution C can be stored at room temperature, and the validity period is 12 months.

Component

Component Number	Component	G1008-20ML	G1008-100ML
G1008-1	PAS solution A	20 mL	100 mL
G1008-2	PAS solution B	20 mL	100 mL
G1008-3	PAS solution C	20 mL	100 mL
Instruction Manual		1 pc	

Preparation

Bring your own Hematoxylin differentiation solution (recommended G1039), Hematoxylin bluing solution (recommended G1040), xylene, gradient ethanol, anhydrous ethanol, neutral gum, etc.

Assay Protocol / Procedures

1. Dewaxing as followed:

Xylene I for 10 min;

Xylene II for 10 min;

100% ethanol I for 5 min;

100% ethanol II for 5 min;

90% ethanol for 5 min;

75% ethanol for 5 min;

Rinsing with tap water;

2. Stain sections with PAS solution B for 10-15 min, rinse three times with distilled water, each time about 10 s.
3. Stain with PAS solutino A (return to room temperature ahead of time for 25-30 min) in the dark, rinse for 5 min.
4. (Optional) Then stain it with PAS solution C for 30s, and rinse with tap water. Treat the slices with Hydrochloric acid solution and Ammonia, each step required washing with water. Then the sections were differentiated by hematoxylin differentiation solution for 3 s and washed with tap water; they were re-blued by hematoxylin bluing solution for 3 s and washed with tap water. This step is for cell nucleus staining.
5. Dehydrate as followed:

100% ethanol I for 5 min;

100% ethanol II for 5 min;

100% ethanol III for 5 min;

Xylene I for 5 min;

Xylene II for 5 min;

Finally seal with neutral gum.

Note

1. PAS solution B can be reused. The dye solution is transparent. If it is obviously yellow, contains impurities, or the staining of the tissue is too light, it needs to be replaced with a new dye solution.
2. PAS solution A needs to be stored at 4°C, take it out before use and return to room temperature before using. It is recommended to replace the dye solution with a new one when the color of the solution is obviously red.
3. Each set of staining solution (20 mL size) can be used to stain approximately 80 sections. Replace the stain with a new one when the tissue or cell coloring is significantly lighter or abnormal.
4. Please wear lab coat and disposable gloves during operation.

Servicebio® Masson Tricolor Staining Kit

Cat No.: G1006

Product Information

Product Name	Cat.No.	Spec.
Masson Tricolor Staining Kit	G1006-20mL	6×20 mL
	G1006-100mL	6×100 mL

Description

Masson's three-color staining method, also known as Masson staining, is a more classic collagen fiber staining method. This method makes use of the different permeability of anionic biological dyes and molecular weights in connective tissues. Dyes with small molecular weights can easily penetrate tissues with dense structures and low permeability, while dyes with large molecular weights can only enter tissues with loose structures and good permeability, so different tissue components present different colors.

This product is a kit, the main components are as follows: A solution is 2.5% Potassium dichromate, B solution and C solution are mixed in equal volume to be Weigert iron hematoxylin dye solution, D solution is Ponceau acid fuchsin, E Liquid is 1% phosphomolybdic acid solution, and F liquid is 2.5% aniline blue solution. After the connective tissue sections stain with Masson's trichrome staining solution, collagen fibers show sky blue to bright dark blue, muscle fibers, cytoplasm, cellulose, and keratinous white show red to purplish red, and red blood cells show light red.

Storage and Handling Conditions

Store at room temperature, valid for 12 months.

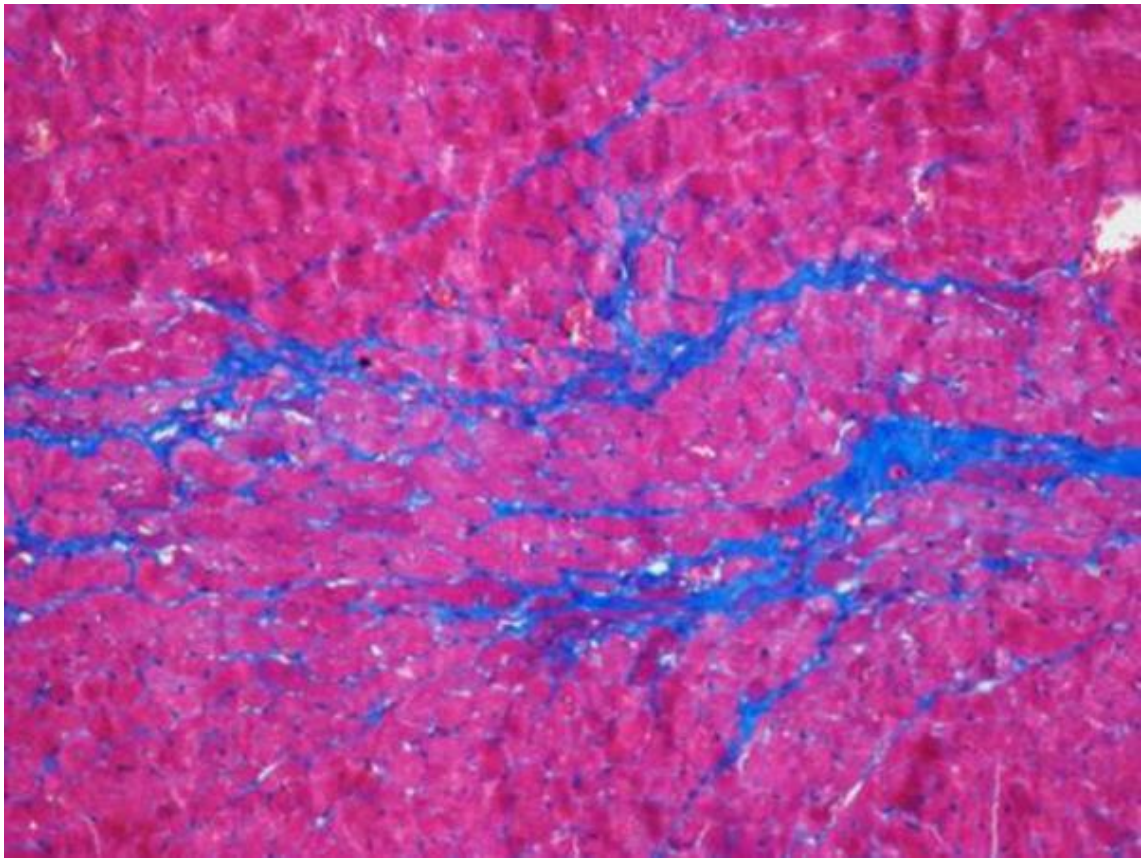
Component

Component Number	Component	G1006-20ML	G1006-100ML
G1006-1	Masson A solution	30 mL	100 mL
G1006-2	Masson B solution	20 mL	100 mL
G1006-3	Masson C solution	20 mL	100 mL
G1006-4	Masson D solution	20 mL	100 mL
G1006-5	Masson E solution	20 mL	100 mL
G1006-6	Masson F solution	20 mL	100 mL
Manual		1 pc	

1. Section preparation: paraffin sections are deparaffinized to water; frozen sections stored at -20°C need to be allowed to stand and return to room temperature.
2. Soak the slices in Masson A solution at room temperature overnight (about 15 hours).
3. Soak the slices in Masson A solution and incubate in a 65° oven for 30 mins. Wash with tap water for 30 s until the yellow color on the tissue fades. At the same time, put Masson D liquid and Masson F liquid in a 65° oven to preheat.
4. Mix Masson B solution and Masson C solution in equal volume (prepared for current use, not pre-prepared and preserved), slice into the mixed solution and soak for 1 min, then rinse with running water.
5. Put the slices into 1% hydrochloric acid alcohol (concentrated hydrochloric acid: absolute ethanol = 1:100) for differentiation for about 1 min,until the nucleus was gray-black, and the background was almost colorless or light gray.
6. Rinse with tap water and drain the excess water on the slices. Dip the slices into Masson D solution for 6 minutes. At this time, the tissues will appear bright red. If the red is too light, the staining time can be prolonged.
7. Drain the slices slightly (not dry slices), and soak in Masson E solution for about 1 min. This step is for differentiation. It is enough to differentiate until the collagen fibers are light red and the fibers are red. The time of E solution can be adjusted according to the degree of staining, usually 1-2 min.
8. After the section is slightly drained of Masson E solution, without water washing, it is directly stained into Masson F solution for 2-30 s. 30 s for bone tissue and 60 s for skin tissue. In the case of frozen sections (8 μm) the staining time needs to be shortened appropriately and adjusted according to the degree of staining.
9. The slices are rinsed and differentiated in three consecutive tanks with 1% glacial acetic acid, each tank is about 7 seconds.The goal is to differentiate the excess aniline blue. The third tank of 1% aqueous glacial acetic acid rinsed in water was examined microscopically to avoid excessive blue color differentiation.
10. The slices are dehydrated in successive three cylinders of absolute ethanol for about 3 s, 5 s, and 5 s. The slices were transparent through xylene for 5 min and sealed with neutral gum.

Note

1. The temperature is low in winter. It is recommended to use Masson A solution, Masson D solution and Masson F solution after heating, and the corresponding dyeing time also needs to be adjusted.
2. After treating the Masson A, the washing time of slices should not be too long, which will lead to the overall weak and dark dyeing effect..
3. Masson E solution can be reused. If the dye solution changes color obviously, it needs to be replaced with a new one.
4. Please wear lab coat and disposable gloves during operation.



Servicebio® Modified Giemsa Staining Kit

Cat. No.: G1079

Product Information

Product Name	Cat. No.	Spec..
Modified Giemsa Staining Kit	G1079-100T	100T

Product Description/Introduction

The modified Giemsa staining solution is composed of azurin, eosin and fluorescent pink B. Azurin is an alkaline dye that can bind to basophilic particles in cells such as nuclear proteins or lymphocyte cytoplasm to be dyed blue-purple; eosin and fluorescent pink B are acidic dyes, which can bind to eosinophilic particles in cells such as cytoplasm and be dyed pink, while neutral particles can bind to eosin, fluorescent pink B and azurin in an equal point state, showing lavender.

This product is modified Giemsa staining solution, adding fluorescent pink B, which can better distinguish the acidic particles in eosinophils. Meanwhile, the staining differentiation solution can also make the staining result more contrast, and can present a clear staining image of cells under a light microscope. It is mainly suitable for cell smear and paraffin/frozen tissue section with good staining effect and clear staining.

Storage and Shipping Conditions

Store at room temperature away from light for 24 months.

Product Content

Component Number	Component	G1079-100T
G1079-1	Modified Giemsa staining stock solution	25 mL
G1079-2	Modified Giemsa staining Diluent	250 mL
G1079-3	Modified Giemsa staining differentiation solution	250 mL
Manual		1 pc

Assay Protocol / Procedures

1. Cell smears (blood, bone marrow and alveolar lavage fluid, etc.):
    1) Smears were taken, dried naturally, and fixed (blood smears were air-dried and fixed by soaking in methanol for 15 min; alveolar lavage fluid and bone marrow smears were air-dried and fixed by soaking in acetone for 1 min);
    2) Take 1 part of modified Giemsa staining stock solution and add 9 parts of the modified Giemsa diluent to mix thoroughly, which is the working liquid;
    3) Add the working liquid, stain at room temperature for 10 min, and use 100-500 μL for each section;
    4) Rinse slowly from one end of the slide with distilled water to remove the stain on the surface of the slide;
    5) Put in oven for drying after microscope inspection;
    6) Slice into clean xylene transparent for 1min and seal with neutral gum.
2. Paraffin/Frozen Tissue Section:
    1) Pretreatment
        a) Paraffin section dewaxing to water: put the slides into environment-friendly dewaxing

solution I and II for 15 minutes each; Anhydrous ethanol I, II and III for 5 minutes each; 75% ethanol for 2 minutes; Water washing for 1 minute;

b) Frozen slices stored at -20℃, removed and restored to room temperature tissue to dry, fixed with methanol for 1min; Freshly cut frozen sections should be dyed after OCT is completely dried to prevent peeling;

2) Take 1 part of modified Giemsa staining stock solution and add 9 parts of the modified Giemsa diluent to mix thoroughly, which is the working liquid;
3) Add the working liquid, stain at room temperature for 10 min; For frozen section, the dyeing time and dilution ratio of stock solution can be adjusted according to the depth of dyeing. The dosage of each section is 100 μL;
4) Rinse slowly from one end of the slide with distilled water to remove the stain on the surface of the slide;
5) Shake off the water and put it into the modified Giemsa staining differentiation solution for 2-3 times;
6) Shake off the water and put it into the pure water 2-3 times;
7) Put in oven for drying after microscope inspection;
8) Slice into clean xylene transparent for 1 min and seal with neutral gum.

Result

Cell Type	Cytoplasmic Color
Mature Red Blood Cell	Pink
Neutrophil	Lavender
Eosinophil	red
Basophil	Blue-Purple
Lymphocyte, Monocyte	Blue-Purple

Note

1. Blood, bone marrow and alveolar lavage fluid smears should be evenly spread, and fixed after natural drying, otherwise the cells are easy to fall off during the staining process.
2. Modified Giemsa staining stock solution is toxic to human body and flammable. Please be careful when operating, and pay attention to effective protection to avoid contact with human body or inhalation of human body.
3. If the dyeing is too light, it can be redyed, and the operation of redyed is the same as that in the manual. If dyed too deep can be soaked in pure water.
4. The optimal dilution ratio of Modified Giemsa staining stock solution can be adjusted according to different tissues or smears.
5. pH value has an impact on cell staining, dyeing slides must be cleaned to avoid acid and alkali pollution affecting the staining results.
6. The working liquid should be prepared and used immediately, otherwise precipitation will occur and affect the dyeing result.
7. Each set of staining solution can be used to stain approximately 100 sections (drop staining). Replace the stain with a new one when the tissue or cell coloring is significantly lighter or abnormal.

Servicebio® Oil Red O solution

Cat No.: G1015-100ML

Product Information

Product Name	Cat.No.	Spec.
Oil Red O solution	G1015-100ML	100 mL

Description

Oil red O, also known as Sudan red 5B, is a fat soluble azo dye. This dye can specifically stain neutral lipids such as triglycerides in cells or tissues, but it is weak for phospholipids and steroids. The basic principle is that oil red O dissolves in lipids to make lipids red to orange red.

The saturated oil red O dye solution of this product is the saturated solution of oil red O. It can be used to dye tissue sections or cells after being diluted with distilled water before use. After dyeing, the fat drops in the tissues are red to orange red.

Storage and Handling Conditions

Storage and transportation under room temperature and away from light, valid for 12 months

Component

Component	G1015-100ML
Oil Red O solution	100 mL
Product Manual	

Assay Protocol

Preparation of working solution: Before use, 6 parts of saturated oil red O dye solution are thoroughly mixed with 4 parts of distilled water, and left to stand overnight at 4 ℃. The next day, filter once with qualitative filter paper, and then filter again at 4 ℃ for 24 hours to obtain the oil red O working solution. In addition, 60% isopropanol is prepared.

I Sample preparation

1. For cells: aspirate the cell culture medium, slowly add PBS (G4202 is recommended) to the edge of the orifice plate to simply clean the cells. Add 4% paraformaldehyde fixative (G1101 is recommended) and fix it at room temperature for 8-10 min, and rinse it twice with PBS.

2. For frozen sections: take out the sections from - 20 ℃ and let them stand at room temperature for 5-10 min to recover to normal temperature.

I Dyeing steps

1. For cells

(1) Add a small amount of 60% isopropyl alcohol into the pore plate to cover the cells for 15-20 seconds, and then suck out 60% isopropyl alcohol and dry the water slightly.

(2) Add oil red O working solution to the orifice plate to cover the cells, and dye them at room temperature in dark for 30 min to remove the dye.

(3) Add 60% isopropanol for rapid differentiation for 3-5 seconds, wash with pure water for 3 times, and each

time for 5 minutes.

(4) (Optional) Add hematoxylin dye solution (**G1004 is recommended**) to dye the nucleus, wash with water, turn blue and then wash with water.

(5) PBS was added to cover the cells and observed under microscope. In case of cell climbing, glycerol gelatin film sealant (**G1402 is recommended**) can be used for slide mounting.

2. For frozen sections

(1) Frozen sections recovered to room temperature were gently immersed in oil Red O working solution and stained for 8-10 min (covered to avoid light).

(2) The sections were taken out, stayed for 3 s, and then immersed in two cylinders of 60% isopropanol for differentiation for 3-5 s.

(3) Sections were immersed in two cylinders of pure water for 10 s each time.

(4) (Optional) The sections were immersed in hematoxylin dye solution to stain the nuclei, washed with water, then returned to blue and washed again. After slightly drying, glycerin gelatin was added to mount the slide.

Note:

1. If it is a frozen section of fresh tissue, the section should be fixed before staining.
2. During the whole operation, pay attention to the gentle action to avoid fat loss or displacement.
3. Samples stained with oil Red O cannot be stored for a long time, and should be observed and photographed as soon as possible.
4. When using glycerin gelatin to mount slides, attention should be paid to avoid bubbles as far as possible. If bubbles are not allowed to press the glass slide or forcibly tear the cover glass after mounting the slides, it will cause fat displacement. The slide can be immersed in warm water at 50-60 °C to allow the cover slide to fall off and then re-mount the slide.
5. This product can be used to stain approximately 80 sections. Replace with new stain when tissue or cell staining is significantly light or abnormal in color.
6. For your safety and health, please wear a lab coat and disposable gloves during operation.

Servicebio® Feulgen Stain Kit

Cat No.: G1048-50ML

Product Information

Product Name	Cat.No.	Spec.
Feulgen Stain Kit	G1048-50ML	4×50 mL

Description

The Feulgen reaction is the traditional way to visualize DNA. The basic principle is that the aldehydes formed by the calculation of DNA combine with Schiff reagent to form a magenta derivative. Feulgen staining is based on this principle. It is specific for DNA, and the nuclei of bacteria or cells can be observed under ordinary light microscopy after staining with this method.

This product Feulgen dyeing solution set contains 4 components. The active component of Feulgen dye solution A is hydrochloric acid, Feulgen dye solution B is colorless fuchsin dye solution, Feulgen dye solution C is sodium sulphite, Feulgen dye solution D is fast green, the concentration is 0.16%. After staining, the nuclear DNA was purplish red, and the cytoplasm and other components were light green

Storage and Handling Conditions

Wet ice transportation; Among them, Feulgen dye solution B needs to be stored at 4℃ away from light, and the rest can be stored at room temperature. It is valid for 12 months.

Component

Component Number	Component	G1048-50ML
G1048-1	Feulgen Stain Solution A	50 mL
G1048-2	Feulgen Stain Solution B	50 mL
G1048-3	Feulgen Stain Solution C	50 mL
G1048-4	Feulgen Stain Solution D	50 mL
Product Manual		

Assay Protocol

1. The paraffin sections are dewaxed to water.

2. After the sections were moistened in Feulgen dye solution A at room temperature, the sections were treated with Feulgen dye solution A at 60℃ for 10-30 min, and then the sections were treated with Feulgen dye solution A at room temperature for 3-5 s. The sections were rinsed 3 times with pure water for 5-10 s each time.

3. After the sections were covered in Feulgen dye solution B and stained for 60-90 min, the sections were removed and directly immersed in Feulgen dye solution C twice, 2 min each time. Then the sections were removed and rinsed with running water for 5 min.

4. Sections were counterstained with Feulgen dye solution D for 5-30 s, and then dehydrated by 3 cylinders of absolute ethanol for 5 s, 5 s, and 30 s, respectively. The dyeing time was adjusted according to the degree of staining.

5. The slices were transparent through xylene for 5 min and sealed with neutral gum.

Note: Prepare absolute ethanol, xylene, neutral gum, etc.

Note:

1. Wear a lab coat and disposable gloves during operation.

Servicebio® Nuclear Fast Red Stain

Cat. No.: G1035-100ML

Product Information

Product Name	Cat.No.	Spec.
Nuclear Fast Red Stain	G1035-100ML	100 mL

Description

Nuclear fast red, CAS number is 6409-77-42. Nuclear fast red can be bonded to the negatively charged nucleus by ionic or hydrogen bonds to stain the nucleus.

The effective component concentration of the nuclear fast red stain is 0.1%, which is often used for nuclear staining. When tissue sections or cells are stained, the nuclei appear red.

Storage and Handling Conditions

Store and transport at room temperature. Valid for 12 months.

Components

Component	G1035-100ML
Nuclear Fast Red Stain	100 mL
Manual	1 pc

Usage

After the paraffin sections were deparaffinized by xylene and washed by gradient ethanol, or after the immunohistochemical reaction was completely developed, they were rinsed with distilled water or tap water, and then the nuclei were stained with Nuclear Fast Red Stain for 5-10 min, and then rinsed by running water, dehydrated and dried by gradient ethanol (70-80%-95%-100%), and then after the xylene was made transparent, they could be sealed with neutral gum.

Note: Provide your own gradient of ethanol, xylene, and neutral gum.

Note

1. If the solution appears precipitation, filtered before use, and it does not affect the staining effect.
2. 100 mL of staining solution can be used to stain approximately 100 sections (drop staining).
3. Please wear 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® Crystal Violet Dye

Cat. No. G1014-50ML

Product Information

Product Name	Cat.No.	Spec.
Crystal Violet Dye	G1014-50ML	50 mL

Description

Crystal violet, also known as Gentian violet, is an alkaline dye that binds to the DNA in the cell nucleus to stain the nucleus.

The concentration of our Crystal Violet Dye is 0.1%, which can be used to stain cells, tissue sections and bacteria to observe cell morphology.

Storage and Handling Conditions

Ship and store at room temperature away from light, valid for is 12 months.

Component

Component Number	Component	G1014
G1014	Crystal violet dye	50 mL

Protocol

1. For adherent cells: cells were fixed with 4% paraformaldehyde (**recommended G1101**) for 10-15 min and washed with water for 3 times, 5 min each. Crystal violet solution was dropped to cover the cells, and the cells were stained at room temperature for 3-10 min (the time was adjusted according to the staining results and requirements). After being fully washed with tap water, the cells could be observed under the microscope.
2. For suspended cells: suspended cells were stained with crystal violet solution in a ratio of 10:1 for 3-10min (the time was adjusted according to the staining results and requirements), and observed under microscope after directly dropped on the slides.
3. For paraffin sections of tissues: the sections were dewaxed and rehydrated, and crystal violet solution was added to cover the tissues, stained at room temperature for 3-10 min (the time was adjusted according to the staining results and requirements). After being fully washed with tap water, the sections could be observed under the microscope.

Note

1. 50 mL of solution can be used to stain approximately 50 sections (drop staining).
2. For your safety and health, please wear lab coat and disposable gloves during operation.

Servicebio® Goldner Trichromatic Staining Solution

Cat. No.: G1064-20ML

Product Information

Product Name	Cat.No.	Spec.
Goldner Trichromatic Staining Solution	G1064-20ML	6×20 mL

Description

Goldner trichromatic staining solution, similar to Masson trichromatic staining, usually refers to staining of nuclei and selectively displaying collagen and muscle fibers. The dyeing principle is related to the size of the anionic dye molecules and the permeability of the tissues. The dyes with small molecular weight can easily penetrate the tissues with dense structure and low permeability, while the dyes with large molecular weight can only enter the tissues with loose structure and high permeability. This product is mainly used for staining bone tissue sections, which can distinguish mineralized bone from non-mineralized bone (osteoid). The collagen fibers in mineralized bone undergo conformational changes during mineralization and deposition of inorganic salts. Thus, after staining, mineralized bone is stained green, non-mineralized bone (osteoid) is stained orange-red, and the nucleus is blue-purple.

Storage and Handling Conditions

Store and transport at room temperature from light; valid for 12 months.

Component

Component Number	Component	G1064-20ML
G1064-1	Goldner Staining Solution A: Hematoxylin Iron Solution A	20 mL
G1064-2	Goldner Staining Solution B: Hematoxylin Iron Solution B	20 mL
G1064-3	Goldner Staining Solution C: Acid Ponceau Fuchsin Solution	20 mL×2
G1064-4	Goldner Staining Solution D: Organge G Solution	20mL
G1064-5	Goldner Staining Solution E: Brilliant Green Solution	20mL
Product Manual		

Assay Protocol

1. Paraffin sections shall be dewaxed and rehydrated according to conventional steps.

2. Before use, mix equal volumes of Goldner staining solution A and Goldner staining solution B according to the dosage. This mixture is ready for use and can be prepared as needed. The sections were stained in the mixture for 20 min, washed with tap water, and then rapidly differentiated with 1% hydrochloric acid alcohol for 2 s. The sections were washed with tap water and distilled water.
3. The sections were stained with Goldner staining solution C for 5-10 min, and then rinsed twice with 0.2% glacial acetic acid for 3-5 s each time.
4. The sections were immersed in Goldner staining solution D for 3 min and observed under a microscope. At this time, the red color of the collagen should fade.
5. The sections were immersed in Goldner dye solution C again for 3-5 min, and rinsed quickly with 0.2% glacial acetic acid twice, 3-5 s each time.
6. Sections were immersed in Goldner solution E staining for 3-5 min, and then rapidly differentiated with 0.2% glacial acetic acid for 3 times, 2 s each time. Then it was rapidly dehydrated with absolute ethanol for 3 s and 5 s, respectively.
7. Transparent sealing: The slices were dehydrated with absolute ethanol for 5 min, transparent with xylene for 5 min, and sealed with neutral gum.

Note

1. The staining degree of Goldner staining solution C and Goldner staining solution D should be controlled according to the staining depth. The collagen part should not be too red, which will affect the coloring of Goldner staining solution D.
2. After Goldner staining solution E staining, attention should be paid to controlling the degree of differentiation to avoid insufficient or excessive differentiation.
3. Wear a lab coat and disposable gloves during operation.

Servicebio® Safranin O-Fast Green Staining Reagent For Bone Tissue

Cat. No.: G1053-100ML

Product Information

Product Name	Cat.No.	Spec.
Safranin O-Fast Green Staining Reagent For Bone Tissue	G1053-100ML	2×100 mL

Description

Saffranine O is a kind of cationic dye that can combine with multiple anions. It can combine with polysaccharide anion groups in cartilage tissues such as chondroitin sulfate and keratin sulfate to make it red. Moreover, the color of saffron O is approximately proportional to the concentration of anions, which can indirectly reflect the content and distribution of proteoglycans in cartilage matrix. If cartilage is damaged, glycoproteins in the cartilage are lost, showing light staining or no staining of saffron O. The acid dye fast green combines with eosinophilic components of tissues to give them a green or blue color. Cartilage and bone were distinguished by saffron - fast - green staining.

This product bone tissue saffron fast green dye solution, saffron dye solution concentration is 0.2%, the concentration of fast green dye solution is 2%. It is suitable for bone tissue staining. After staining, the cartilage is red to bright red, and the osteogenesis is green to blue-green.

Storage and Handling Conditions

Store and transport at room temperature, valid for 12 months.

Component

Component Number	Component	G1053-100ML
G1053-1	Safranin O solution	100 mL
G1053-2	Fast-Green solution	100 mL
Product Manual		

Assay Protocol

1. Paraffin sections were dewaxed to water: sections were successively dewaxed by xylene for 10 min, followed by fresh xylene for 10 min, absolute ethanol for 5min, fresh absolute ethanol for 5min, 90% ethanol for 5min, 75% ethanol for 5min, and washed with tap water.
2. Fast green staining: The sections were stained with fast green staining solution for 1-5 min. The excess

staining solution was washed with tap water until the cartilage was colorless. The sections were rapidly treated with 1% hydrochloric acid for 10-15 s, and then washed with tap water.

3. Saffron staining: Sections were stained with saffron staining solution of bone tissue for 5-10 s, and then rapidly dehydrated by four cylinders of absolute ethanol for 3-5 s each time. After the fourth dehydration, microscopic examination showed that the cartilage was red and the background was colorless.

4. Transparent sealing: Sections were transparent through xylene for 5 min, then transparent through fresh xylene for 5 min, and then sealed with neutral gum.

5. Microscopic examination, image acquisition and analysis.

Note: Prepare your own xylene, gradient ethanol, neutral gum, etc.

Note

1. The section should not be stained with saffron for too long, otherwise it is difficult to differentiate, resulting in mixed color with fast green. If you need to re-stain, you can soak in tap water to remove the fast green color, acidic differentiation solution (**recommended G1039**) to remove the saffrane color, and then stain again after all the colors are decolorized.

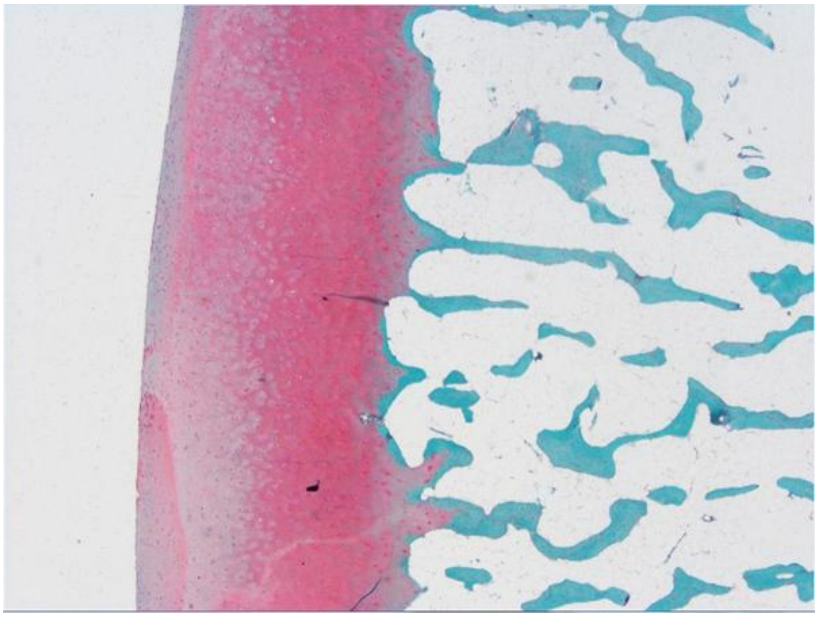
2. The excess dye can be removed by washing after fast green staining. Under the microscope, the bone formation is obviously green, and the cartilage is very light or colorless.

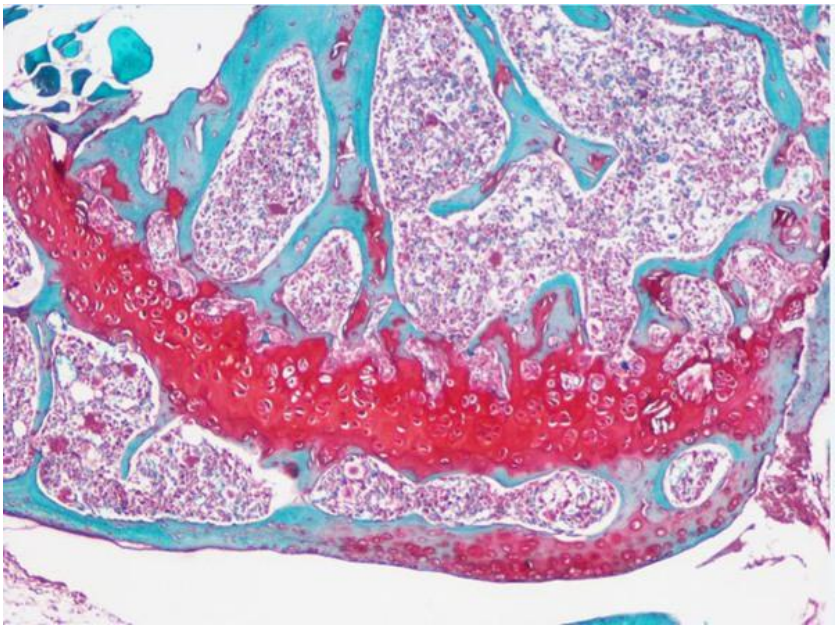
3. Each set of staining solution can be used for staining (dip-staining) approximately 50 sections. Replace the stain with a new one when the tissue or cell coloring is significantly lighter or abnormal.

4. Wear a lab coat and disposable gloves during operation.

For Research Use Only!

Image:





The cartilage is bright red and the osteogenesis is green.

Servicebio® TRAP Staining Kit

Cat. No.: G1050-50T

Product Information

Product Name	Cat.No.	Spec.
TRAP Staining Kit	G1050-50T	50 T

Description

Tartrate resistant acid phosphatase (TRAP) is the signature enzyme of osteoclasts, which is specifically distributed in osteoclasts. TRAP staining kit is used to visualize osteoclasts in tissues. The basic principle is that under acidic conditions containing tartaric acid, anti-tartaric acid phosphatase TRAP can hydrolyze naphthol AS-BI phosphate, and the resulting naphthol AS-BI binds to hexaazole-pararosaniline to form a non-water-soluble burgundy substance deposited in the enzyme-activated in-situ site, which realizes the chromatography and localization of anti-tartaric acid phosphatase.

The basic components of the product: the main components of the reaction buffer are acetic acid buffer and potassium sodium tartrate, pH about 5.0; pararosaniline solution, containing pararosaniline; the main component of sodium nitrite solution is 4% sodium nitrite; AS-BI phosphate substrate solution, the main component is 20 mg/mL naphthol AS-BI phosphate. After staining with this product, TRAP in osteoclasts was Burgundy and localized in the cytoplasm. According to the amount of 300 μL per tissue spot on the slice, the kit can do more than 50 TRAP stainings.

Storage and Handling Conditions

Wet ice transportation; Store at 2-8℃ and avoid light, in which AS-BI phosphate substrate solution is stored at -20℃, valid for 12 months.

Component

Component Number	Component	G1050-50T
G1050-1	Acetic acid buffer solution & Potassium sodium tartrate	20 mL
G1050-2	Pararosaniline solution	1 mL
G1050-3	Sodium nitrite solution	1 mL
G1050-4	AS-BI Phosphate Solution	1 mL
Product Manual		

Assay Protocol

Preparation Before Experiment

Prepare TRAP working fluid:

(1) Take 50 μL pararosaniline solution (G1050-2) and 50 μL sodium nitrite solution (G1050-3) and mix them in a clean centrifuge tube to get hexaazole-pararosaniline solution;

(2) Add 100 μL AS-BI phosphate substrate solution (G1050-4) to 100 μL hexaazole-pararosaniline solution in step 1, and blow and aspirate several times to fully;

(3) Absorb 1.8 mL reaction buffer (G1050-1) and add it to the mixture solution in step 2 mix thoroughly;

(4) The mixed liquid in step 3 is filtered through a needle filter (0.45 μm stream filter film) to obtain the TRAP working liquid.

Note: Be sure to prepare the working solution in the order it belongs to. About 200-300 μL of working solution is required for each tissue point, which should be prepared according to the amount used and used as needed to avoid waste.

Paraffin Sectioning Procedure (For Reference)

1. Paraffin sections are dewaxed to water and washed in pure water for several minutes.
2. Put the sections in a tissue chemical pen circle (with a certain amount of pure water to prevent the drying of sections) in a wet box, and incubate the sections with pure water at 37℃ for 2 h.
3. After the section is incubated, pour out pure water, add filtered TRAP working solution to cover the tissue, and place it at 37 °C in the dark for 20-30 min.
4. (**Optional, prepare your own reagents**) Counterstain the nuclei: pour the incubation solution and wash it with hematoxylin dye solution to stain the nuclei.
5. Dehydrate, transparent, and seal with neutral gum.

Operation Procedure of Cell Slide (For Reference)

1. Cell fixation: remove the cell culture medium by suction, add 4% paraformaldehyde (**G1101 recommended**) and fix for 15-30 min, then wash with distilled water for 3 times.
2. Cell membrane rupture: Cells were covered with 0.2% Triton X-100 solution for 20-30 min and gently washed with distilled water for 3 times.
3. Incubation and staining: TRAP working solution was added to the cell well plate to cover the cells, and the cells were incubated at 37℃ for 15-20 min in the dark, and washed with distilled water for 3 times.
4. (**Optional, prepare your own reagents**) Nuclear counterstaining: Blot the incubation solution and wash it with hematoxylin staining solution for nuclear staining.
5. Add an appropriate amount of absolute ethanol for dehydration, take out the cover glass in the well plate, dry it with a hair dryer, and seal it upside down on the clean glass slide with neutral gum.

Servicebio® Toluidine Blue Dye Solution

Cat No.: G1032-100ML

Product Information

Product Name	Cat.No.	Spec.
Toluidine Blue Dye Solution	G1032-100ML	100 mL

Description

Toluidine Blue is a quinone imine basic staining, which can combine with acidic substances in tissue cells to achieve tissue staining. The nuclei and Nissl's body in neurons is stained into blue with toluidine blue, which can be used for the preliminary pathological diagnosis of Nissl body; The cytoplasm of mast cells contains heparin and histamine, and the cartilage contains chondroitin sulfate. These substances have metachromatic properties and are stained into purplish red with toluidine blue. Therefore, toluidine blue staining can be used to observe the distribution and abnormal changes of mast cells and the morphological structure of cartilage, such as tide lines; plant tissues can be stained and observed, the xylem, ducts, sieve tubes and other structures, the xylem are stained into blue-green and the cellulose cell wall is stained into blue-purple.

The active ingredient concentration of the toluidine blue dye solution of this product is 0.5%, which can be used for the staining of conventional animal and plant tissue sections.

Storage and Handling Conditions

Transport at room temperature; Valid for 18 months.

Component

Component	G1032
Toluidine Blue Dye Solution	100 mL
Manual	1 pc

Assay Protocol / Procedures

1. Paraffin sections were dewaxed to water;
2. Toluidine blue stain: Put the tissue sections into the toluidine blue dye solution for 2-5 minutes, wash slightly with tap water to remove excess dye.
   a. For plant tissue: Controll the degree of staining under the microscope,and according to the degree of staining, use 0.1% glacial acetic acid for proper differentiation. If the degree of coloring is appropriate and does not require differentiation, the slices are dried in a 60°C oven.
   b. For animal tissue: Wash animal tissue sections with water and differentiated with 0.1% glacial acetic acid. The degree of differentiation was observed microscopically, and differentiation was sufficient until the background was light blue and the coloration of the nidus, cartilage, and mast cells was evident. Rinse with tap water to terminate differentiation and then dry it in an oven at 60℃.

3. Transparent and mount: Sections were transparent to xylene for 10 min and then sealed with neutral gum.

Note

1. The washing time of the stained sections should not be too long, otherwise the color will fade easily.
2. Sections must be completely dried prior to transparent sealing so that any remaining tiny droplets of water do not interfere with observation.
3. Staining Solution is reusable. 100 mL of Staining Solution can be used to stain (dip or drop) approximately 350 sections. Replace the stain with a new one when the tissue or cell coloring is significantly lighter or abnormal.
4. Please wear lab coat and disposable gloves during operation.

Servicebio® Alcian Blue Dye Solution

Cat No.: G1027

Product Information

Product Name	Cat.No.	Spec.
Alcian Blue Dye Solution	G1027-100ML	100 mL

Description

Alcian blue is any member of a family of polyvalent basic dyes which belongs to cationic dye and initially was used for dyeing textile fibers. This dye is soluble in water and has a blue color due to the presence of copper in the molecule. It is a dye specific for acid mucins, which can bind to acidic groups to form salt bonds and stain the tissues with compounds containing anionic groups, such as acid mucosubstances and acidic mucins. Alcian blue can make acid mucopolysaccharides in the cytoplasm appear blue, while the neutral mucins are not stained. It is often used in paraffin section technology to distinguish carboxyl mucus from sulphate mucus, identify mucinous epithelial tumors, and mark the cartilage in bone tissue. A general method uses an alcian blue solution at pH 2.5 which can stain many acidic mucins and acid mucosubstances. The alcian blue solutions with even lower pH value stain only strongly acidic sulfated mucosubstances (pH 0.4) or acidic sulfated mucosubstances (pH 1.0).

This product containing 1% alcian blue dye, pH is about 2.5, which can be used for cartilage tissue staining. It can also be used in combination with PAS, which can distinguish between acid mucins and neutral mucins, with acid mucins appearing blue and neutral mucins red.

Storage and Handling Conditions

Room temperature, the shelf life is 12 months.

Component

Component Number	Component	G1027
G1027	Alcian blue dye solution	100 mL

Assay Protocol

1. Paraffin slides dewaxed as follow: Two changes of pure xylene for 20 min, two changes of pure ethanol for 5min; 75 % ethanol for 5 min; keep slides in tap water.
2. The slides stained in Alcian blue dye for 10~15 min, and then washed in running water.
3. The slides stained in Nuclear fast red (G1035) for 3 min, and then washed in running water (optional).
4. Three changes of pure ethanol for 5min, two changes of pure xylene for 5 min transparent and then coverslip with neutral resin.
5. Observed under microscope and took images.

Note

1. The staining time can be adjusted according to the degree of staining. The longer the staining time, the darker the blue tissue.
2. If it is necessary to observe the tissue morphology, the Nuclear fast red dye solution (G1035) can be used for redyeing and the section background color will be red.
3. Please wear lab coat and disposable gloves during operation.

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