Растворы для морфологического окрашивания, специальные пятна, ч. 1 (окрашивание патологических отложений, клеток, образцов животных и растений, микробиологическое, кальциевых узелков)

Технические характеристики

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Servicebio® Congo Red Dye Solution

Cat No.: G1056-250ML

Product Information

Product Name	Cat.No.	Spec.
Congo Red Dye Solution	G1056-250ML	2×250 mL

Description

Amyloid substance is an extracellular eosinophilic substance with no fixed shape, mainly composed of proteins, most of which are arranged in reverse β- Folding layer structure, such amyloid substance can exist in different tissues and organs, and the disease is called amyloidosis. Congo red can be used for coloration of amyloid in living and tissue sections. The basic principle is that amyloid has a high affinity for Congo red dye. Its hydroxyl group combines with the amino group of Congo red to make amyloid red. Congo red staining is a classical method of amyloid staining.

This product is composed of Congo red dye solution and alkaline differentiation solution, which can be used to dye amyloid substances. After staining, the amyloid plaque material presents light pink to orange red cloud or patch shape, and under polarized light, the amyloid material also presents yellow green double refraction.

Storage and Handling Conditions

Store and transport at room temperature; valid for 18 months.

Component

Component Number	Component	G1056-250ML
G1056-1	Congo Red Staining	250 mL
G1056-2	Alkaline Differentiation Solution	250 mL
Product Manual		

Assay Protocol

If it is necessary to re stain the nucleus, prepare hematoxylin staining solution, hematoxylin differentiation solution and hematoxylin blue returning solution. Prepare ethanol, xylene, neutral gum, etc.

1. The paraffin section shall be dewaxed and rewatered according to the routine steps.
2. Congo red staining: slice into Congo red dye solution for overnight impregnation, and then wash with tap water for 2 minutes.
3. Differentiation: The slices were differentiated into positive amyloid plaques with obvious background after alkaline differentiation solution, and then washed with tap water.
4. (Optional) Nucleation staining: the slices were stained with hematoxylin dye solution (**G1004**) for 1 min, differentiated with hematoxylin differentiation solution (**G1039**), washed with tap water, and the hematoxylin bluing solution (**G1040**) turned blue, washed with running water.

5. Dehydration and mounting: slice is dehydrated by anhydrous ethanol for three times, each time for 5 minutes; the xylene is transparent twice for 5min each time. Drop a neutral gum to mount the slide.

Note:

1. Alkaline differentiation solution should be sealed to prevent solvent volatilization from affecting differentiation effect. Use it as soon as possible after opening.
2. The differentiation time varies according to section thickness and tissue type. After differentiation, the differentiation should be terminated by washing in time to prevent excessive differentiation, and sufficient washing should be ensured to completely remove the differentiation solution.
3. Each set of staining solution can be used for staining (dip-staining) approximately 90 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.

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® Alizarin Red S Soluiton

Cat. No.: G1038-100ML

Product Information

Product Name	Cat.No.	Spec.
Alizarin Red S Soluiton	G1038-100ML	100 mL

Description

Alizarin red S, also known as alizarin sodium sulfonate, is an anthraquinone derivative, can chelate with calcium carbonate or calcium phosphate in the calcium salt to form an orange-red complex, can be used to stain a small amount of calcium salt deposits.

This product alizarin red dyeing solution S, the active ingredient concentration is 2%, the dyeing solution pH 4.2. It can be used to stain calcium salt deposits in tissues, and has a good staining effect on pathological calcifications such as calcification of tuberculous caseous necrosis foci, calcification of diseased artery wall in aortic atherosclerosis, calcification of dead parasite eggs and other foreign bodies. After staining, the calcium salt deposits are red or orange, with a light red or almost colorless background. This product has reliable staining results for a small amount of calcium salt deposits. It is not suitable for the samples with rich calcium salt.

Storage and Handling Conditions

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

Component

Component	G1038-100ML
Alizarin Red S Soluiton	100 mL
Product Manual	

Usage (Take Paraffin Section as an Example)

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 washed with tap water.
2. Drop alizarin red S dye solution onto the section to completely cover the tissue, and stain for 5-10 min. The staining time was determined according to the calcium salt content, and it was timely observed under the microscope when the calcium salt was dark orange-red.

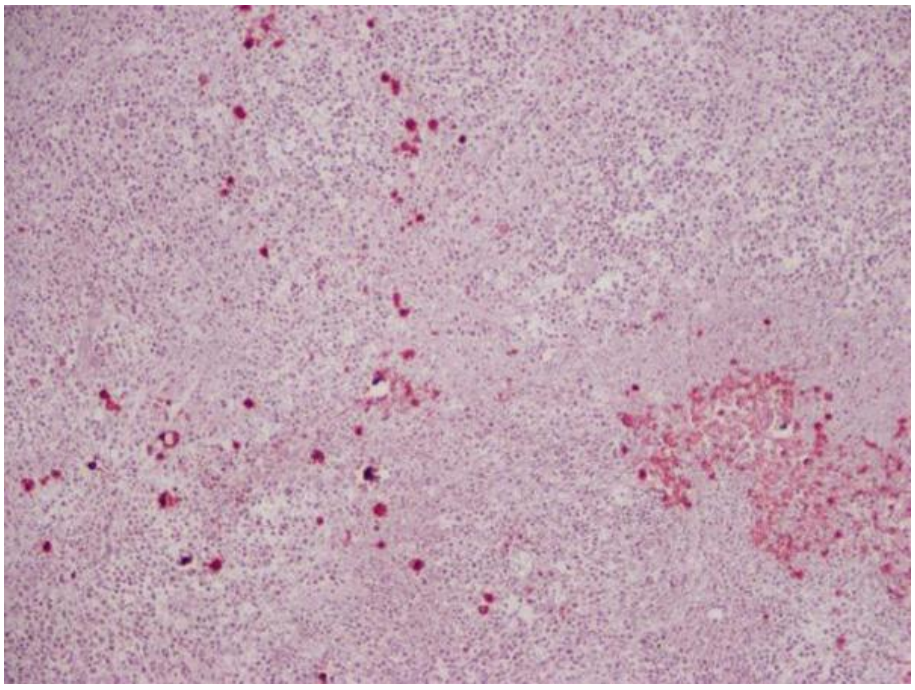
3. Pour the dye solution and wash with tap water until the glass slide is colorless.
4. (Optional) The nucleus is counterstained with the complexing solution such as fast green or hematoxylin, washing.
5. Slice and bake in oven at 65℃ for 4 h.
6. Cut into fresh xylene and make clear for 5-10 min. Seal the slices with neutral gum.

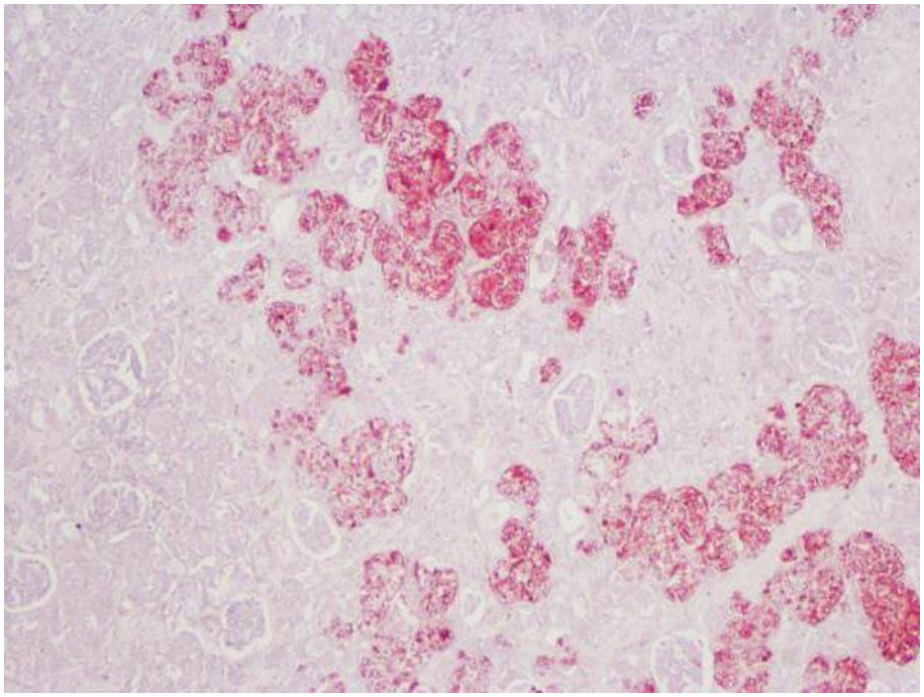
Note: Prepare gradient ethanol, xylene, neutral gum, etc.

Note

1. The stained sections cannot be dehydrated with absolute ethanol; otherwise, the orange color will turn to dark red and part of the calcium salt will be lost.
2. Each 100 mL of Staining Solution can be used to stain (dip) approximately 60 sections. Replace with new Staining Solution when tissue or cell staining is significantly lighter or abnormal in color.
3. Wear a lab coat and disposable gloves during operation.

Image：





Calcium deposits are reddish, with a reddish or almost colorless background.

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® 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:

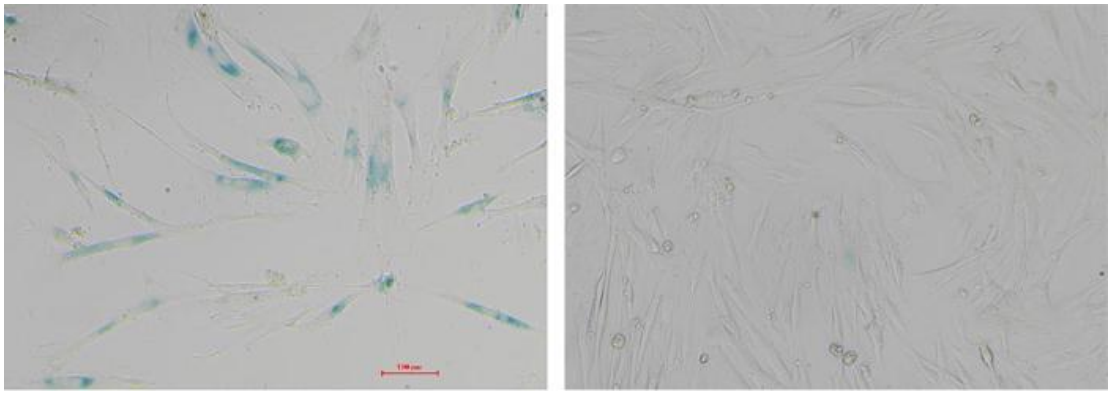


Fig.1 WI-38 cells were stained with β-galactosidase kit. The left picture shows senescence WI-38 cells without division and proliferation ability but still alive. After staining, the positive staining cells were more than 95%. The image on the right shows newly resuscitated WI-38 cells (early passage)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

Servicebio® 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® TTC Staining Solution（0.5%，Animals）

Cat No.: G1017-100ML

Product Information

Product Name	Cat.No.	Spec.
TTC Staining Solution	G1017-100ML	100 mL

Description

TTC (2, 3, 5-triphenyltetrazolium chloride), a lipid-soluble light-sensitive complex, can be used to detect seed viability or to detect ischemic infarction in mammalian tissues. The basic principle is that TTC is a proton acceptor of pyridine-nucleoside structural enzyme system in respiratory chain, and can react with dehydrogenase in normal tissues to form water insoluble red Triphenylformazan (TTF) compounds. Normal tissues and viable seeds are rich in dehydrogenase, which can react with TTC and appear red. If the tissue dies from ischemia or seed viability declines, it cannot react with TTC, and the tissue cannot be stained or the staining is very light. The degree of tissue ischemia infarction or seed vigor can be determined according to the staining site and depth.

The concentration of TTC in TTC staining solution of this product is 0.5%, which is suitable for the staining of ischemic infarction in animal tissues. The area of normal tissue is red, and the area of ischemic infarction is white.

Storage and Handling Conditions

Wet ice transportation; Store at 2-8℃ away from light, valid for 3 months.

Component

Component	G1017-100ML
TTC Staining Solution (0.5%, Animals)	100 mL
Product Manual	

Assay Protocol

Sample Pretreatment: The ischemic infarcted tissues were taken immediately after the animals were sacrificed and placed in a clean petri dish. The tissues were wrapped with OCT embedding agent (**G6059 is recommended**) and flash-frozen at -20℃ for 30-60 min. If frozen sections could not be prepared in time, they should be stored at -80℃. Before staining, the tissue should be thawed slightly at room temperature and then sliced into sections with a very sharp blade with a thickness of 2-3 mm. Ensure that the sections are

neat.

Staining Steps

1. TTC staining solution was preheated at 37℃ for 30 min.
2. Put the tissue sections into a clean container (be careful not to make the tissue be squeezed), add TTC staining solution to soak the tissue, and put the container in a 37℃ water bath to avoid light for about 30 min. During the period, the tissue was gently shaken every 10 min to contact the staining solution evenly, and the staining situation was observed in time.
3. When the tissue appears dark red, pour out TTC dye solution (which can be recycled), and slowly add fixing solution (**G1101 is recommended**) into the container to soak the tissue to terminate the staining reaction. Samples should be stored away from light.
4. Before taking photos, remove the tissue, blot the fixation solution with filter paper, and take photos with camera as needed.

Note

1. Quick sampling is required to ensure fresh samples and maximize enzyme activity.
2. The stained samples are easy to fade, so photos should be taken as soon as possible.
3. Wear a lab coat and disposable gloves during the 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:

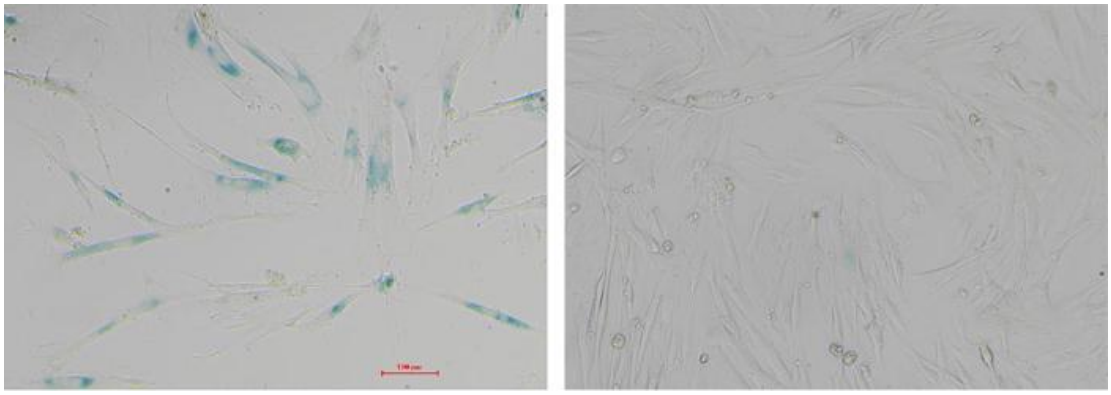


Fig.1 WI-38 cells were stained with β-galactosidase kit. The left picture shows senescence WI-38 cells without division and proliferation ability but still alive. After staining, the positive staining cells were more than 95%. The image on the right shows newly resuscitated WI-38 cells (early passage)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

Servicebio® Aniline Blue Stain (10%)

Cat No.: G1071-100ML

Product Information

Product Name	Cat.No.	Spec.
Aniline Blue Stain (10%)	G1071-100ML	100 mL

Description

This product is a 10% aniline blue aqueous solution, which can be used to display fungi in plant tissues, and can also be used to dye the morphological structure of plant tissues. After staining, the fungal hyphae were blue filamentous, and the tissue background was light blue.

Storage and Handling Conditions

Store and transport at room temperature; valid for 6 months.

Assay Protocol

1. Dewaxing the paraffin slices to water: (This step is for reference only) Put the slices into xylene I for 20 min - xylene II for 20 min - absolute ethanol I for 5 min - absolute ethanol II for 5 min - 75% ethanol for 5 min, and wash them with tap water.
2. Aniline blue staining: dye the slices with aniline blue dye (10%) for 5-10 min, wash them quickly with tap water, and bake the slices at 60 ℃.
3. Dehydration seal: the slices are transparent with xylene for 5 min, and sealed with neutral gum.

Note:

1. This product can be reused several times and should be kept sealed to prevent evaporation of the active ingredient.100 mL of staining solution can be used for staining (immersion staining) of approximately 500 sections. When the tissue or cell coloring is significantly lighter or the color is abnormal, please replace with new staining solution.
2. Please wear a lab coat and disposable gloves during operation.

Servicebio® Lodine-Potassium Lodide Dye Solution I-KI Dye Solution

Cat No.: G1070-50ML

Product Information

Product Name	Cat.No.	Spec.
Lodine-Potassium Lodide Dye Solution I-KI Dye Solution	G1070-50ML	2 x 50 mL

Description

This product can be used to display starch in plant tissues or to identify pollen fertility. The principle is that iodized starch is formed when iodized potassium iodide solution interacts with starch, which shows a special blue reaction. Iodized potassium iodide solution is a classical method to identify starch and an important reagent for plant histochemical identification. This product contains two components: iodine-potassium iodide staining solution and plant solid green staining solution. After staining, the starch grains were blue or blue-black, and the cell walls were green. If used to identify pollen fertility, fertile pollen grains are round, large and plump, with a uniform dark blue color.

Storage and Handling Conditions

Store and transport at room temperature; the iodine-potassium iodide staining solution should be stored sealed away from light and valid for 12 months.

Component

Component Number	Component	G1070-50ML
G1070-1	Iodine-potassium iodide staining solution	50 mL
G1070-2	Plant solid green dye	50 mL
Product Manual		

Assay Protocol

1. Plant tissue section observation of starch granules (prepared anhydrous ethanol, xylene, neutral gum, etc.)

(1) Paraffin sections dewaxed to water: histochemical strokes encircle tissues,

(2) Iodine potassium iodide staining: circle the tissues with histochemical strokes, and add iodine potassium iodide solution to cover the tissues for 5-10 minutes. Quickly wash twice for 2 seconds each time.

(3) (Optional) Fast Green fixation staining: the slices were dehydrated quickly with 2 cylinders of absolute ethanol for 10 seconds each time. Immerse in the plant fast green dye for 10 s. The slices were dehydrated rapidly by 3 cylinders of absolute ethanol for 10 seconds each time.

(4) Transparent and mounting: xylene is transparent for 5 min, and the section is sealed with neutral gum. It is very easy to fade, and it is recommended to take a mirror inspection as soon as possible and take photos to save the results.

2. Identification of pollen fertility (taking rice pollen as an example)

(1) Anther collection: when the rice is heading, the spikelets flowering at the early stage of the rice spike are taken and fixed in 75% ethanol, and stored at 4 ℃. Generally, it is required to take the upper, middle and lower parts of the rice spike, and the anther length is more than 2/3 of the length of the glume as the

mature anther.

(2) Staining microscopy: use pointed tweezers to take the anthers and put them on the slide, add 1-2 drops of iodine potassium iodide staining solution to cover the anthers, use tweezers to crush the anthers, so that the pollen grains can be fully released. Then cover the cover glass, gently press the cover glass with tweezers, and let it stand for 2-3 minutes. Observe under a 10 fold microscope.

(3) Generally, fertile pollen grains are round, large and plump, dark blue, and evenly colored; There are four types of sterile pollen, including pollerless type, typical abortion, round abortion and dye abortion. Pollen free type refers to no pollen or only residual pollen wall observed under microscope; The typical abortion means that the pollen is not stained and its shape is irregular, such as triple tube, polygon, etc; Round abortion refers to the round appearance of pollen grains without stained starch grains; Pollen abortion refers to that most pollens are normal in morphology, but their coloration is shallow or uneven, and some pollens are deeply stained, but their grain shape is obviously different from that of normal fertile pollens.

Note:

1. After using the reagent, please tighten the cap in time to prevent solvent volatilization.
2. Each set of staining solution can be used to stain approximately 400 sections. Replace the stain with a new one when the tissue or cell coloring is significantly lighter or abnormal.
3. Wear a lab coat and disposable gloves during operation.

Servicebio® PAS+Naphthol Yellow S Staining Solution

Cat No.: G1068-100ML

Product Information

Product Name	Cat.No.	Spec.
PAS+Naphthol Yellow S Staining Solution	G1068-100ML	3×100 mL

Description

This product is used to stain protein and starch grains in plant tissues. Under the action of periodate acid, starch in plant tissue and carbohydrate in cell wall exposed aldehyde group, aldehyde group combined with Schaffer reagent to produce red reaction, that is, PAS staining. Proteins in plants are often stored in plant cells in the form of aleurone granules, which are solid and surrounded by a membrane to form spheroidal granules. Naphthol yellow S reacts with proteins to produce yellow reactions. Therefore, PAS staining combined with naphthol yellow can simultaneously display starch grains and proteins in plant tissues. After staining, plant starch granules are purplish red, cell walls are purplish red, and proteins appear yellow.

Storage and Handling Conditions

Wet ice transportation; Keep away from light, in which the PAS dye solution A should be stored at 4℃, and the rest of the reagents can be stored at room temperature, and the validity period is 12 months.

Component

Component Number	Component	G1068-100ML
G1068-1	PAS dye solution A	100 mL
G1068-2	PAS dye solution B	100 mL
G1068-3	Naphthol yellow S dye solution	100 mL

Assay Protocol

1. Paraffin sections are dewaxed to water.
2. Immerse the slices in PAS dye B for 10 min, and soak them in pure water for 3 times, 10 s each time.
3. The slices were immersed in PAS dye A (recovered to room temperature in advance) for 20-30 min in dark, washed with running water for 5 min, and microscopic examination showed that the cell wall and the starch granules in the cells were purple red.
4. The slices were immersed in the Naiphenol Yellow S dye solution for 5 min, washed quickly with distilled water for 2 s, and dehydrated quickly with three cylinders of absolute ethanol for 3 s, 5 s, and 30 s respectively.
5. The slices were transparent with xylene for 5 min and sealed with neutral gum.

Note:

1. PAS dye solution B can be reused. The dye solution is transparent, and should be replaced with a new dye solution if it shows obvious yellow color, has impurities, or is too light in tissue staining.
2. The PAS dye solution A should be stored at 4℃, and then taken out and restored to room temperature

before use. When the color of the solution is obviously red, it is recommended to replace the new dye.

3. Each set of staining solution can be used to stain approximately 400 sections. Replace the stain with a new one when the tissue or cell coloring is significantly lighter or abnormal.

4. Protein coloring is easy to fade, and the sections cannot be stored for a long time. It is recommended to observe and take photos as soon as possible to save the results.

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

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® Plant Superoxide Anion Stain (NBT)

Cat No.: G1023-100ML

Product Information

Product Name	Cat.No.	Spec.
Plant Superoxide Anion Stain (NBT)	G1023-100ML	3×100 mL

Description

Plant tissues produce a variety of reactive oxygen species (ROS) under stress conditions. ROS activity is very large and extremely unstable, so the detection of ROS usually depends on its final products. Superoxide anion is a kind of reactive oxygen species, belonging to a kind of oxygen-containing free radical, can reduce NBT (nitro-blue tetrazolium) to water insoluble blue formazan compound, thereby locating superoxide anion in tissues. It is also known as the NBT method based on basic principles. This product is based on the above principle for superoxide anion staining in plant living tissue. It is generally applied to the whole staining of tender root tips, leaves, etc. After staining, the site of superoxide anion accumulation is blue to dark blue.

Storage and Handling Conditions

Wet ice transportation; Store at 2-8℃ away from light, valid for 12 months.

Component

Component number	Component	G1023-100ML
G1023-1	NBT	50 mg
G1023-2	Tris Buffer Solution (pH 7.4)	100 mL
G1023-3	NBT Sample Solution	2×100 mL
Product Manual		

Assay Protocol

1. Reagent preparation: 50 mg NBT was fully dissolved in 100 mL Tris buffer (pH 7.4) to obtain NBT staining working solution, which was stored at 4℃ under light and effective within one week.
2. Collect seedlings or root tips, wash them with pure water, and blot the excess water on filter paper. Immerse the plant seedlings or root tips in NBT staining working solution at room temperature and avoid light for 2-6 h until the positive parts appear dark blue, and the rest parts are light blue or nearly colorless or the color of the plant itself (according to the degree of youth of the plant, the degree of color development adjusts the dyeing time).
3. Carefully remove plant seedlings or leaves with tweezers, rinse with pure water for 3-5 times, put them on filter paper to blot out excess water, and then immerse them in 95% ethanol for 3-16 h at 40℃ to remove the chlorophyll or light blue background of plant seedlings or leaves. Fresh 95% ethanol can be replaced many times during treatment.
4. Take out seedlings or leaves with tweezers, dip them into pure water and rinse them 3-5 times, put them

on filter paper to drain the excess water, transfer the samples into an appropriate amount of NBT sample preservation solution and soak for 30 min, then take out the photos. Samples can be stored in the solution at room temperature for one week.

Note

1. After preparing the NBT staining solution, it should be stored at 4℃ in the dark and used within a week. Storage time is too long, will affect the color.
2. Since any external factors may stimulate plant stress to produce superoxide anion, plant samples should be collected fresh and stained as soon as possible. Negative and positive blank control group is recommended.
3. Take photos as soon as possible to save the results after sample staining.
4. Wear a lab coat and disposable gloves during operation.

Servicebio® Safranin O-Fast Green Staining (Plant)

Cat No.: G1031-100ML

Product Information

Product Name	Cat.No.	Spec.
Safranin O-Fast Green Staining (Plant)	G1031-100ML	2×100 mL

Description

Safranin, also known as Safranin O, is an alkaline dye that shows lignified, corked, and keratinized tissue in vascular plants. Fast green is an acid dye that can display cellulose cell tissue containing plasma. The combination of saffron and fast green staining is widely used in animal and plant tissue and cell staining.

This product is suitable for the tissue section staining of roots, stems and leaves of plants. The main components are as follows: The effective component concentration of plant saffron staining solution is 1%, and the effective component concentration of plant fast green staining solution is 0.5%. After staining with this product, plant ducts, xylem, lignified cell walls and nuclei were red, sieve tubes, parenchyma cells, cell walls containing cellulose and cytoplasm were green.

Storage and Handling Conditions

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

Component

Component Number	Component	G1031-100ML
G1031-1	Plant saffron staining solution	100 mL
G1031-2	Plant fast green staining solution	100 mL
Product Manual		1 pc

Assay Protocol

1. The paraffin sections are dewaxed to water.
2. **Saffron staining**: the sections were stamped with plant saffron staining solution and soaked for 2 min. The excess dye was quickly washed off with tap water. Then the sections were rapidly differentiated into 50%, 70% and 80% ethanol, 3-5 s each time.
3. **Fast green staining**: The sections are stained with plant fast green staining solution for about 6-30 s (it can be adjusted according to the thickness of the sections and the degree of staining), and the sections are removed and slightly filtered to dry the staining solution. The sections were rapidly dehydrated with absolute ethanol three times, 5-10 s each time.
4. **Transparent sealing**: Sections were transparent through xylene for 5 min, and then sealed with neutral gum.

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

1. Paraffin sections should be fully dewaxed to avoid affecting the staining effect.

2. The washing time after saffron dyeing should not be too long, otherwise the saffron is easy to fade.

3. This product can be reused several times. After use, it is sealed and stored to prevent volatile active ingredients. Each set of staining solution can be used for staining (dip-staining) approximately 500 sections. When the tissue coloring is significantly light, it is recommended to replace a new dye solution.

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

Servicebio® Plant Hydrogen Peroxide Staining Liquid (DAB)

Cat No.: G1022-100ML

Product Information

Product Name	Cat.No.	Spec.
Plant Hydrogen Peroxide Staining Liquid (DAB)	G1022-100ML	3 x 100 mL

Description

Plant tissues produce a variety of reactive oxygen species (ROS) under stress conditions. ROS activity is very large and extremely unstable, so the detection of ROS usually depends on its final products. Hydrogen peroxide is a type of reactive oxygen species. Catalyzed by catalase, hydrogen peroxide can rapidly react with DAB (3, 3-diaminobenzidine tetrahydrochloride) to form a brown-red compound, so as to locate hydrogen peroxide in tissues. According to the basic principle, it is also called DAB method. Based on the above principle, this product is used for hydrogen peroxide staining in plant living tissues. It is generally applied to the tender root tips, leaves and other overall staining, after staining the site of hydrogen peroxide accumulation is brown to dark brown.

Storage and Handling Conditions

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

Component

Component Number	Component	G1022-100ML
G1022-1	DAB	100 mg
G1022-2	Phosphate Buffer (pH 3.8)	100 mL
G1022-3	DAB Sample Retention Solution	2×100 mL
Product Manual		

Assay Protocol

1. Reagent preparation: 100 mg DAB was fully dissolved in 100 mL phosphate buffer to obtain DAB staining working solution, which was stored at 4℃ under light and effective within one week. Note: DAB is sensitive to light and needs to be shielded from light during the dissolution process. If it is difficult to dissolve, the dissolution can be promoted by ultrasonic and magnetic stirring.
2. Collect seedlings or root tips of plants under stress (such as heavy metals), wash them slightly with pure water, and put them on filter paper to drain the excess water. Plant seedlings or root tips were immersed in DAB staining working dye solution at room temperature and kept away from light for 2-6 h until the positive part appeared dark brown, and the rest parts were nearly colorless or showed the color of the plant itself. (Adjust the dyeing time according to the degree of youth and color development of plants)
3. Carefully remove the plant seedlings or leaves with tweezers, immerse them in pure water and rinse them back and forth 3-5 times, put them on filter paper to blot out the excess water, and then immerse them in 95% ethanol at 40℃ for 3-16 h. The purpose is to remove the chlorophyll of the plant seedlings or leaves

themselves, and the fresh 95% ethanol can be replaced many times during decolorization.

4. Take out seedlings or leaves with tweezers, immerse them in pure water and rinse them back and forth for 3-5 times, put them on filter paper to blot out excess water, transfer the samples into an appropriate amount of DAB sample preservation solution and soak them for 30 min, then take photos. Samples can be stored in the solution at room temperature for one week.

Note:

1. After the DAB staining working solution is prepared, it should be stored at 4℃ and kept away from light, and used within one week. Storage time is too long, will affect the color.
2. Since hydrogen peroxide is easy to decompose and any external factors may stimulate plant stress to produce hydrogen peroxide, plant samples need to be collected fresh and stained as soon as possible. Negative and positive blank control group is recommended.
3. Take photos as soon as possible to save the results after sample staining.
4. Wear a lab coat and disposable gloves during operation.

Servicebio® Aniline Blue Stain (10%)

Cat No.: G1071-100ML

Product Information

Product Name	Cat.No.	Spec.
Aniline Blue Stain (10%)	G1071-100ML	100 mL

Description

This product is a 10% aniline blue aqueous solution, which can be used to display fungi in plant tissues, and can also be used to dye the morphological structure of plant tissues. After staining, the fungal hyphae were blue filamentous, and the tissue background was light blue.

Storage and Handling Conditions

Store and transport at room temperature; valid for 6 months.

Assay Protocol

1. Dewaxing the paraffin slices to water: (This step is for reference only) Put the slices into xylene I for 20 min - xylene II for 20 min - absolute ethanol I for 5 min - absolute ethanol II for 5 min - 75% ethanol for 5 min, and wash them with tap water.
2. Aniline blue staining: dye the slices with aniline blue dye (10%) for 5-10 min, wash them quickly with tap water, and bake the slices at 60 ℃.
3. Dehydration seal: the slices are transparent with xylene for 5 min, and sealed with neutral gum.

Note:

1. This product can be reused several times and should be kept sealed to prevent evaporation of the active ingredient.100 mL of staining solution can be used for staining (immersion staining) of approximately 500 sections. When the tissue or cell coloring is significantly lighter or the color is abnormal, please replace with new staining solution.
2. Please wear a lab coat and disposable gloves during operation.

Servicebio® Gram Staining Kits

Cat No.: G1065-20ML

Product Information

Product Name	Cat.No.	Spec.
Gram Staining Kits	G1065-20ML	4×20 mL

Introduction

Gram staining is a differential staining method widely used in microbiology, which can be used to distinguish Gram-positive bacteria (G+) from Gram-negative bacteria (G-). The basic principle is based on the different chemical components of bacterial cell walls. After primary staining with crystal violet and mordant with iodine, bacteria form water-insoluble crystal violet-iodine complexes in the cell wall. Gram-positive bacteria have thick cell walls, do not contain lipids, are rich in peptidoglycan and are cross-linked to form a dense grid structure. When treated with ethanol, the peptidoglycan mesh shrinks, which can block the crystal violet-iodine complex. It remains in the cell wall, giving it the purple color of crystal violet. Gram-negative bacteria, on the other hand, have thin cell walls, low peptidoglycan content and loose cross-linking. When exposed to ethanol, the lipid-rich outer membrane dissolves, a large gap appears in the cell wall, and the crystal violet-iodine complex flows out, so the cells become colorless after ethanol decolorization. At this point by safranin counterstaining, Gram-negative bacteria will be stained red.

Gram staining solution contains four components, namely ammonium oxalate crystal violet staining solution, 1% iodine solution, destaining solution and fuchsin staining solution. After staining, gram-positive bacteria appear purple to blue-purple, and gram-negative bacteria appear red.

Storage and Handling Conditions

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

Component

Component number	Component	G1401-20ML
G1065-1	Ammonium oxalate crystal violet staining solution	20 mL
G1065-2	1% iodine solution	20 mL
G1065-3	destaining solution	20 mL
G1065-4	Fuchsin staining solution	20 mL
Instruction	1	1

Assay Protocol / Procedures

1. Dewax the paraffin section to water; dry the bacterial smear naturally or warm it with an alcohol lamp to dry and fix it.
2. Initial dyeing: Add crystal violet staining solution dropwise to cover the sample for 10-30 s, wash with water, and spin dry.
3. Mordant dyeing: Add 1% iodine solution dropwise to cover the sample for 1-1.5 min, wash with water, and spin dry.
4. Decolorization: dropwise add decolorization solution to rinse the tissue from one end of the slide for decolorization, until the decolorization solution that flows down no longer appears purple, and immediately wash with water to remove the decolorization solution. Take care to control the destaining time.
5. Counterstaining: add Fuchsin staining solution dropwise for 20-60 s, then wash with water. Blot the edges with filter paper.
6. Bacterial smears can be observed directly by microscopy. After drying at 60 °C, the tissue sections were rapidly dehydrated in absolute ethanol for three times, 1 s, 3 s, and 5 s, respectively. Then, it was transparentized with xylene for 5 min, and finally sealed with neutral gum for microscopic examination.

Note

1. Tighten the cap in time after using the reagent to prevent the solvent from volatilizing.
2. The bacterial smear is too thick, the crystal violet staining time is too long, and the decolorization is insufficient, which may lead to false positive results. It is recommended to do both negative and positive bacterial control groups.
3. Each set of staining solution can be used to stain approximately 30 sections (drop staining).
4. Please wear lab coat and disposable gloves during operation.

Ver. No.: V3.0-202311

Servicebio® Acid-fast Staining Solution

Cat No.: G1047-100ML

Product Information

Product Name	Cat.No.	Spec.
Acid-fast Staining Solution	G1047-100ML	3×100 mL

Description

This product is suitable for staining acid-fast bacteria and can be used to distinguish acid-fast bacteria from non-acid-fast bacteria. The basic principle is that there is a layer of lipids surrounding peptidoglycan on the surface of acid-resistant bacteria such as tuberculosis bacteria and Mycobacterium leprae, so it is not easy to stain when stained, and once the stain is promoted by some methods, it is difficult to be decolorized by acid decolorizing agent, hence the name acid-fast staining.

This set of acid-fast staining solution is suitable for detecting acid-fast bacteria in tissues. After staining, the nuclei were blue and acid-fast bacteria were purplish red.

Storage and Handling Conditions

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

Component

Component Number	Component	G1047-100ML
G1047-1	Acid-fast Staining Solution A: Carbonic acid-magenta solution	100 mL
G1047-2	Acid-fast Staining Solution B: Acidic decolorizing solution	100 mL
G1047-3	Acid-fast Staining Solution C: Hematoxylin solution	100 mL
Product Manual		

Assay Protocol

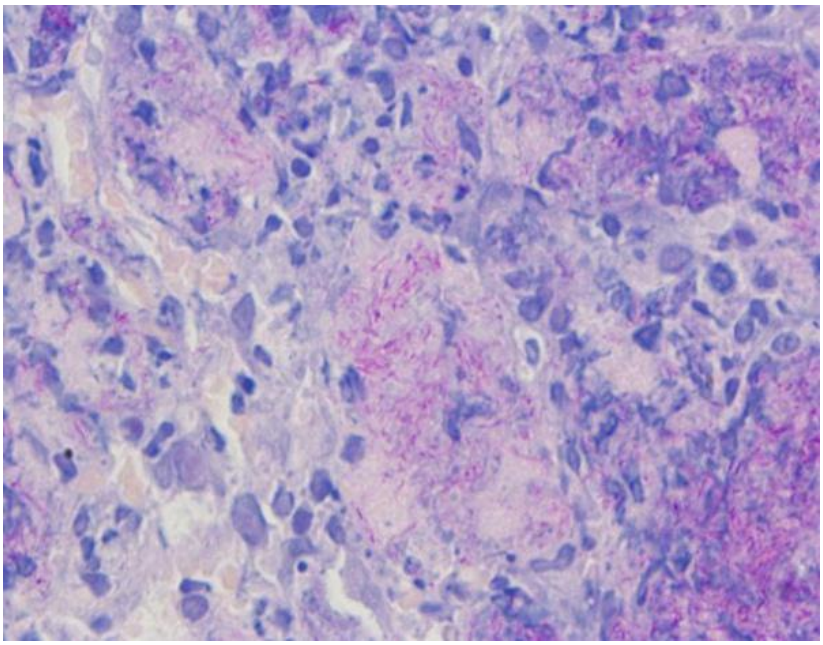
Prepare hematoxylin differentiation solution (**G1039**), hematoxylin bluing solution (**G1040**), xylene, ethanol, neutral gum, etc.

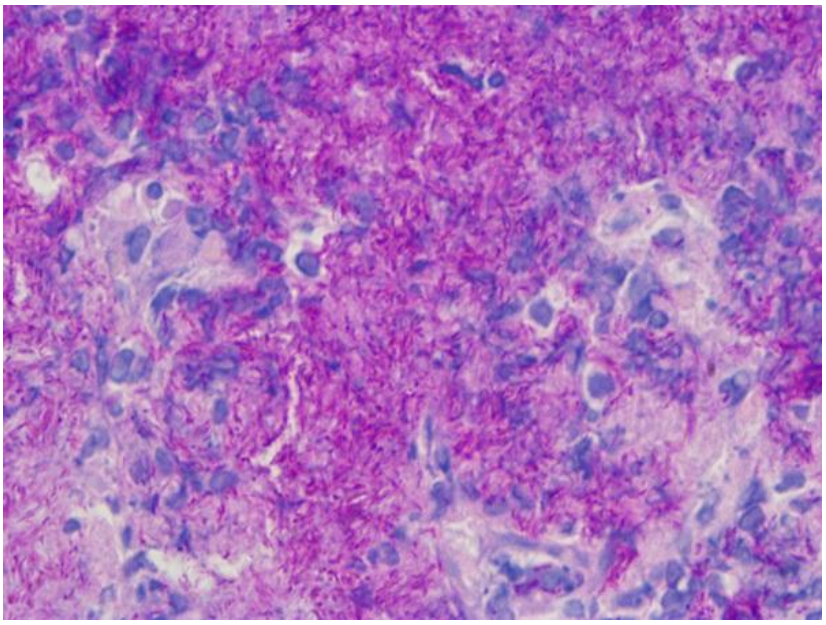
1. Paraffin sections are dewaxed to water.
2. Sections into acid-fast staining solution A, cover and soak for 30 min, and rinse with running water for 2 min.
3. The sections enter the acid-fast staining solution B for 1-2 seconds, and then it is washed with water. Repeat the steps of differentiation and washing, and observe with microscope in time. The acid-fast bacteria can differentiate into purple red, and the tissue background is basically colorless.
4. The slices were stained with acid-fast staining solution C for 30 seconds, then differentiated with hematoxylin differentiation solution for 2 seconds, washed with tap water, and then blued with hematoxylin bluing solution for 3-5 seconds, washed with tap water.
5. The sections were dehydrated by 3 cylinders of absolute ethanol for 5 min each, then transparent by xylene for 5 min, and sealed with neutral gum.

Note:

1. The staining time of acid-fast staining solution A can be appropriately prolonged when the room temperature is low in winter.
2. Tighten the cap in time after using the reagent to prevent volatilization of active ingredients or product deterioration.
3. Each set of staining solution can be used for staining (dip staining) approximately 80 sections. Please 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.

Images:





Acid-fast bacteria are purplish red with blue nuclei.

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® 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® 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® Alizarin Red S Soluiton

Cat. No.: G1038-100ML

Product Information

Product Name	Cat.No.	Spec.
Alizarin Red S Soluiton	G1038-100ML	100 mL

Description

Alizarin red S, also known as alizarin sodium sulfonate, is an anthraquinone derivative, can chelate with calcium carbonate or calcium phosphate in the calcium salt to form an orange-red complex, can be used to stain a small amount of calcium salt deposits.

This product alizarin red dyeing solution S, the active ingredient concentration is 2%, the dyeing solution pH 4.2. It can be used to stain calcium salt deposits in tissues, and has a good staining effect on pathological calcifications such as calcification of tuberculous caseous necrosis foci, calcification of diseased artery wall in aortic atherosclerosis, calcification of dead parasite eggs and other foreign bodies. After staining, the calcium salt deposits are red or orange, with a light red or almost colorless background. This product has reliable staining results for a small amount of calcium salt deposits. It is not suitable for the samples with rich calcium salt.

Storage and Handling Conditions

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

Component

Component	G1038-100ML
Alizarin Red S Soluiton	100 mL
Product Manual	

Usage (Take Paraffin Section as an Example)

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 washed with tap water.
2. Drop alizarin red S dye solution onto the section to completely cover the tissue, and stain for 5-10 min. The staining time was determined according to the calcium salt content, and it was timely observed under the microscope when the calcium salt was dark orange-red.

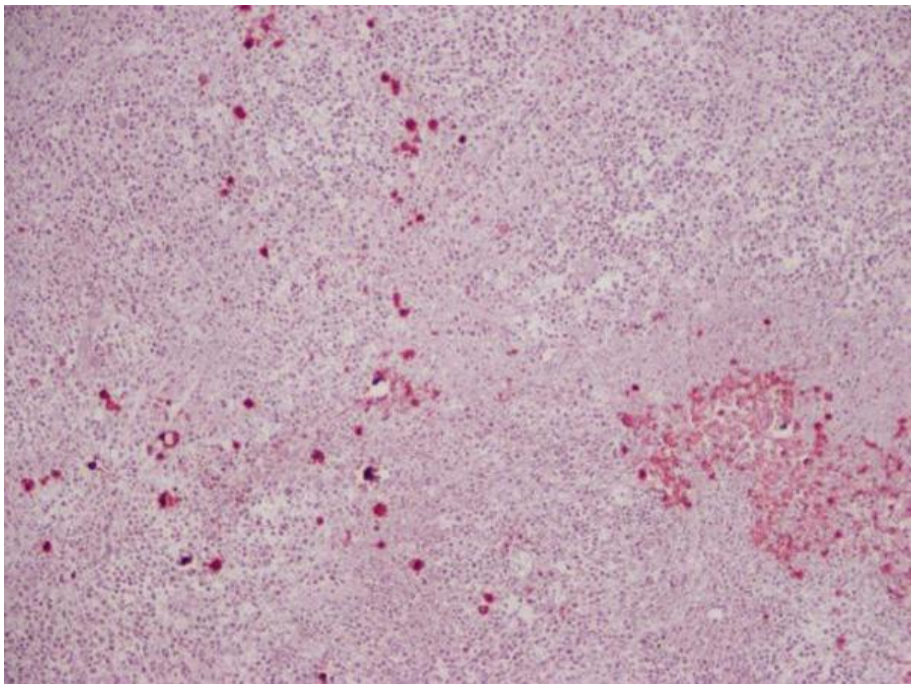
3. Pour the dye solution and wash with tap water until the glass slide is colorless.
4. (Optional) The nucleus is counterstained with the complexing solution such as fast green or hematoxylin, washing.
5. Slice and bake in oven at 65℃ for 4 h.
6. Cut into fresh xylene and make clear for 5-10 min. Seal the slices with neutral gum.

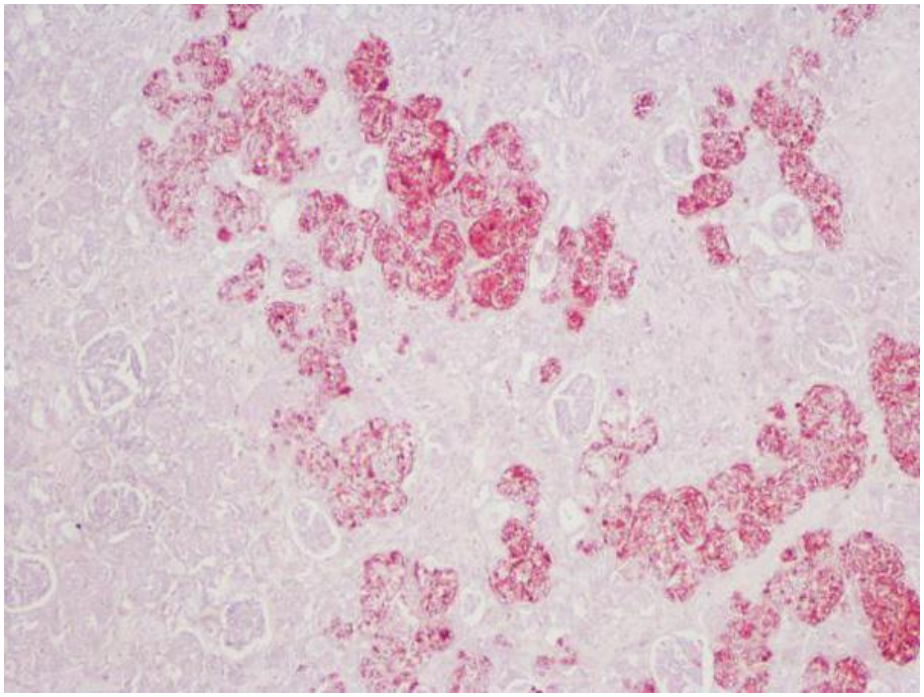
Note: Prepare gradient ethanol, xylene, neutral gum, etc.

Note

1. The stained sections cannot be dehydrated with absolute ethanol; otherwise, the orange color will turn to dark red and part of the calcium salt will be lost.
2. Each 100 mL of Staining Solution can be used to stain (dip) approximately 60 sections. Replace with new Staining Solution when tissue or cell staining is significantly lighter or abnormal in color.
3. Wear a lab coat and disposable gloves during operation.

Image：





Calcium deposits are reddish, with a reddish or almost colorless background.

По вопросам продаж и поддержки обращайтесь:

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Ангарск (3955)60-70-56
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