HITO
Biotec in situ detection

Simple Solution for Your Research

Hito Reticulin OptimStain™ Pap-Pen Kit

[Catalog Number: HTKCS0102P]

An easy to use silver staining system
for the morphological characterization
of the Reticular Fiber

User Manual
and
Material Safety Data Sheet

FOR IN VITRO RESEARCH USE ONLY

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I. Introduction

The reticulin silver stain is commonly used to demonstrate reticular fibers. The method developed by Gordon and Sweet made further improvement on the reticulin silver stain. However, these reticulin staining procedures use relatively large volume of staining solutions, are costly, time -consuming, and difficult to achieve consistent results.

Hito Reticulin OptimStain™ Kit offers a simple solution to these problems. This kit is easy to use with simplified procedures. The users can process slides in small quantities of solutions. The staining step can be controlled slide by slide to achieve optimal staining and differentiation for each slide. This kit delivers stable and improved staining quality, with minimal overstains, background and artifacts when used properly.

Hito Reticulin OptimStain™ Kit has been tested on the liver, spleen, pancreas, lung and other tissues from several species of animals and proven to be sensitive for demonstrating the morphological details of reticular fibers. It is a simple solution for your research.

II. Kit Contents

Store Hito Reticulin OptimStain™ Kit

at 4°C in the dark

Kit Contents

Solution-1	20 ml
Solution-2	20 ml
Solution-3	20 ml
Solution-4A	10 ml
Solution-4B	10 ml
Solution-4C	10 ml
Solution-5	20 ml
Solution-6	20 ml
Solution-7	20 ml
Solution-8	20 ml
Hito Aqua Barrier PAP Pen	1
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Note

Before using Hito Reticulin OptimStain™ Kit, please make sure you have the following **Required Equipment / Materials** in your lab (not included in the kit):

1. Cryostat or Microtome, dry ice, O.C.T. compound, iso-pentane, ethanol, xylene, double distilled or deionized water, Light microscope, Slide and coverslips
2. Staining jars for slides wash
3. Resinous mounting medium

III. Tissue Preparation

For Frozen Section

1. Prepare perfusion system.
2. Prepare animal for infusion by administering a lethal dose of anesthesia. Monitor it until the point when the animal fails to respond to pinching of the foot.
3. Cut the skin of the mouse from the abdomen to the top of the thorax. Open the abdominal wall below the ribcage. Lift the sternum with tweezers and cut the diaphragm. Then cut away the lower part of the ribcage to partially expose the heart.
4. Quickly insert needle of infusion set into left ventricle. Clamp the needle in place.
5. Begin perfusion of PBS very slowly (i.e., 10 to 15 ml/min). After the perfusion system begins pumping the PBS, immediately cut the inferior vena cava to allow an escape route for the blood and perfusion fluid.
6. Perfuse PBS at a moderate to rapid rate (<20 ml/min) and continue until the effluent runs clear, which may require 25 to 50 ml of solution.
7. After the effluent runs clear, stop the pump and introduce 4% PFA into the infusion set line running into the animal. Perfuse 50-100 ml 4% PFA at a moderate to slow rate.
8. Remove and transfer tissue into 4% PFA, store at 4°C. Replace 4% PFA after 24 hours, and continue to store at 4°C for 24 hours.
9. Transfer the tissue into 30% sucrose solution, store at 4°C. Replace 30% sucrose solution after 24 hours, and continue to store at 4°C for 24 hours, until the tissue sinks into the sucrose solution.
10. Place ~300 to 500 ml isopentane in a metal container large enough to hold a corresponding sieve-like basket. Place the metal container with the isopentane in dry ice for 15 to 30 min, until the temperature of the isopentane reaches -70°C.

11. Place the tissue briefly on absorbent paper to remove excess solution.
12. Place the tissue in OCT compound in a base mold on the mesh bottom of the sieve-like basket.
13. Slowly immerse the basket with the tissue in the cooled isopentane for 30 sec. to 1 min.

Note

The time of immersion is absolutely critical; it must be long enough to result in complete freezing of the tissue, but not so long that the tissue cracks. It may be necessary to test various times to determine the one that is optimal to meet these criteria.

14. Wrap the dried, frozen tissue block in aluminum foil and store at -70°C until sectioning is performed.
15. Set the cryostat chamber temperature at **-17°C**.

Note

The -17°C setting is satisfactory in most cases, but may need optimization for different cryostat and tissue types in order to cut sections smoothly and keep integrity.

16. Place specimen holder / cryostat chuck on dry ice and add embedding matrix or water on the surface of the specimen holder / chuck. As the embedding matrix or water begins to freeze, place the frozen tissue block into it so that the tissue block adheres to the specimen holder / chuck.
17. Slowly cut the tissue into sections (15-30 µm thickness) on a cryostat with the chamber temperature set at -17°C.
18. Mount the sections on the Hito Super-Safe Slide.
19. Air dry slides (30 minutes) at room temperature. Dried sections should be processed as soon as possible but may be stored in a slide box at room temperature for one year.

For Paraffin Tissue Section

1. Prepare perfusion system.
2. Prepare animal for infusion by administering a lethal dose of anesthesia. Monitor it until the point when the animal fails to respond to pinching of the foot.
3. Cut the skin of the mouse from the abdomen to the top of the thorax. Open the abdominal wall below the ribcage. Lift the sternum with tweezers and cut the diaphragm. Then cut away the lower part of the ribcage to partially expose the heart.
4. Quickly insert needle of infusion set into left ventricle. Clamp the needle in place.
5. Begin perfusion of PBS very slowly (i.e., 10 to 15 ml/ min). After the perfusion system begins pumping the PBS, immediately cut the inferior vena cava to allow an escape route for the blood and perfusion fluid.
6. Perfuse PBS at a moderate to rapid rate (<20 ml/min) and continue until the effluent runs clear, which may require 25 to 50 ml of solution.
7. After the effluent runs clear, stop the pump and introduce 4% PFA into the Infusion set line running into the animal. Perfuse 50-100 ml 4% PFA at a moderate to slow rate.
8. Remove and transfer tissue into Hito Bouin's Plus Solution or 4% PFA store at 4°C. Replace Hito Bouin's Plus Solution or 4% PFA after 24 hours, and continue to store at 4°C for 24-48 hours.
9. After fixation, dehydrate the tissue in a graded ethanol/ water series at room temperature: (for 0.5 cm^3 tissue)
    - Tap water for 2 hours
    - 50% ethanol for 2 changes of 2 hours each
    - 70% ethanol for 2 changes of 2 hours each
    - 95% ethanol for 2 changes of 1.5 hour each
    - 100% ethanol for 2 changes of 1 hour each

10. Replace ethanol with xylene for 2 changes, each 45 minutes at room temperature.
11. Immerse the tissue in the paraffin wax (56-58°C), 2 changes, 1.5 hour each.

Note

The time of immersion is absolutely critical; it must be long enough to remove xylene in the tissue, but not so long that the tissue stiffens. It may be necessary to test various times to determine the one that is optimal to meet these criteria.

12. Embed tissues in paraffin blocks
13. Turn on the water bath and check that the temperature is 45°C. Use fresh deionized water. Insert the block into the microtome chuck. Set the dial to cut 7-15 μm sections. Cut sections and pick them up with forceps or a fine paint brush and float them on the surface of the water bath. Float the sections onto the surface of Hito Super-Safe Slide.
14. Place the slides with paraffin sections in a 60°C oven for 2 hours (so the wax just starts to melt) to bond the tissue to the glass. Slides can be stored in slide box at room temperature.

IV. Staining Procedure

1. Add 600µl Solution-4A into 2ml Eppendorf microtube. Use a pipet to add 10 µl of Solution-4C to the micro-tube and mix well. A brown color precipitate will appear. Keep adding Solution-4C 10 µl a time and mix well until the brown precipitate just disappears.
2. Add 600µl Solution-4B into the same microtube and mix well. Black precipitate should appear.
3. Use a pipet to add 10 µl of Solution-4C to the micro-tube and mix well. Keep adding Solution-4C 10 µl a time and mix well until the black precipitate is almost completely dissolved. The mixture solution is referred to as Solution-4. Save solution-4 for Step 11 below.

Note

Avoid adding too much Solution-4C which will greatly reduce the staining sensitivity. If too much Solution-4C is added, it can be fixed by adding 10 µl of Solution-4B to the microtube and mix well. Keep adding Solution-4B and mix well until the black precipitate just reappear.

4. Deparaffin / defat and rehydrate the sections by placing the slides in xylene, 100% ethanol, 95% ethanol, 75% ethanol, 50% ethanol and then in double distilled water with 2 changes in each solvent, and 3 minutes during each change.
5. Remove excess liquid from the slides with a paper towel. Draw a loose circle with Hito Aqua Barrier PAP Pen (provided in the kit) around the rehydrated sections. After the Hito Aqua Barrier PAP Pen circle dries, use a pipet to drop Solution-1 onto each section within the Pap-Pen circle. Fully cover the sections by Solution-1 and incubate for 5 minutes .
6. Rinse slides in distilled water twice.
7. Use a pipet to drop Solution-2 onto each section within the Pap-Pen circle. Fully cover the sections by Solution -2 and incubate for 1-2 minutes.
8. Rinse slides in distilled water, 3 times, 20 seconds each.

9. Use a pipet to drop Solution-3 onto each section within the Pap-Pen circle. Fully cover the sections by Solution -3 and incubate for 15 minutes.
10. Rinse slides in distilled water, 5 times, 20 seconds each.
11. Use a pipet to drop Solution-4 onto each section within the Pap-Pen circle. Fully cover the sections by Solution -4 and incubate for 1 minute.
12. Rinse slides briefly in distilled water.
13. Use a pipet to drop Solution-5 onto each section within the Pap-Pen circle. Fully cover the sections by Solution -5 and incubate for 1 minute.
14. Rinse slides in distilled water, 5 times, 1 minute each.
15. Place the slides in the in distilled water until the next step.
16. Using a pipet, drop Solution-6 onto each section within the Pap-Pen circle. Fully cover the sections by Solution -6 and incubate for 30 seconds - 3 minutes under the microscope and check for color change. Once a golden brown tissue color is changed to grey, immediately stop the reaction by placing the slides back into the distilled water.
17. Rinse slides briefly in distilled water.
18. Use a pipet to drop Solution-7 onto each section within the Pap-Pen circle. Fully cover the sections by Solution -7 and incubate for 1-2 minutes.
19. Rinse slides in distilled water twice.
20. Using the dropping bottle, drop Solution-8 onto each section within the Pap-Pen circle. Fully cover the sections by Solution-8 and incubate for 1-2 minutes.
21. Rinse the slides in double distilled water twice, 1 minutes each.
22. Rinse the slides in 50% ethanol, 75% ethanol, 1 minute each.

23. Rinse the slides in 95% ethanol, with 2 changes, 2 minutes during each change.
24. Dehydrate the slides in 100% ethanol, with 2 changes, 3 minutes during each change.
25. Clear in xylene, 2 times, 4 minutes each. Apply coverslip over sections using xylene based resinous mounting medium.
26. Allow to dry. The slide can be viewed after drying by bright field microscopy.

V. References

1. Gordon, H. and H. H. Sweets (1936). "A Simple Method for the Silver Impregnation of Reticulum." Am J Pathol 12(4): 545-552 541.
2. Lillie, R. D. (1977). H.J. Conn's Biological stains : a handbook on the nature and uses of the dyes employed in the biological laboratory.

VI. Material Safety Data Sheet (MSDS)

Date Updated: 7/29/2016
Version 1.4

1. Product and Company Information

Product Name	Hito Reticulin OptimStain™ PAP-Pen Kit
Product Number	HTKCS0102P
Brand	Hitobiotec
Company Address	Hitobiotec Corp. P.O.Box 7528 Kingsport, TN 37664 USA
Technical Phone:	423-520-6880
Emergency Phone:	423-520-6880

2. Composition and Information on Ingredient

Substance Name	**CAS #**	**SARA 313**
Hito Reticulin OptimStain™ Kit	None	No

Ingredient Name	**CAS #**	**SARA 313**
WATER	7732-18-5	No
Silver Nitrate	7761-88-8	Yes
Paraformaldehyde	30525-89-4	No
Sodium Thiosulfate	10102-17-7	No
PROPRIETARY COMPONENT(S)	None	No

3. Hazards Identification

EMERGENCY OVERVIEW

Harmful by inhalation or in contact with skin or eyes. Possible risk of irreversible damage to skin, mucous membranes, eyes, blood, kidneys and digestive, respiratory, reproductive and central nervous systems.

HMIS RATING

HEALTH: 2 FLAMMABILITY: 0 REACTIVITY: 0

NFPA RATING

HEALTH: 2 FLAMMABILITY: 0 REACTIVITY: 0

Potential Health Effects

Inhalation	May be harmful if inhaled. Causes respiratory tract irritation.
Skin	May be harmful if absorbed through skin. Causes skin irritation.
Eyes	Causes eye burns, eye irritation.
Ingestion	Toxic if swallowed. Causes burns.

4. FIRST AID MEASURES

General advice

Consult a physician. Show this safety data sheet to the doctor in attendance. Move out of dangerous area.

If inhaled

If breathed in, move person into fresh air. If not breathing give artificial respiration

In case of skin contact

Take off contaminated clothing and shoes immediately. Wash off with soap and plenty of water. Consult a physician.

In case of eye contact

Continue rinsing eyes during transport to hospital.Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed

Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

5. FIRE-FIGHTING MEASURES

Flammable properties

Flash point	no data available
Ignition temperature	no data available

Suitable extinguishing media

Use water spray, alcohol-resistant form, dry chemical or carbon dioxide.

Special protective equipment for fire-fighters

Wear self contained breathing apparatus for fire fighting if necessary.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions

Use personal protective equipment. Avoid dust formation. Avoid breathing dust. Ensure adequate ventilation. Evacuate personnel to safe areas.

Environmental precautions

Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.

Methods for cleaning up

Pick up and arrange disposal without creating dust. Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Handling

Perform experiment in a properly functioning chemical hood, which is vented to the outside. Wear glasses and disposable gloves while handling kit reagents. Wash hands thoroughly after performing the test.

Storage

Keep container tightly closed in a dry and well-ventilated place. Store at room temperature, preferably in a cool place.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection

Where risk assessment shows air-purifying respirators are appropriate use a full-face respirator with multipurpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection

Handle with gloves.

Eye protection

Safety glasses with side-shields conforming to EN166

Hygiene measures

Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	liquid

Safety data

pH	no data available
Melting point	no data available
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Water solubility	no data available

10. STABILITY AND REACTIVITY

Storage stability

Stable under recommended storage conditions. Avoid Light.

Materials to avoid

Strong oxidizing agents, metals

Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Hydrogen chloride gas, Mercury/mercury oxides, Potassium oxides, Chromium oxides.

11. TOXICOLOGICAL INFORMATION

Acute toxicity	Refer to component MSDS
Irritation and corrosion	Refer to component MSDS
Sensitisation	Refer to component MSDS

Signs and Symptoms of Exposure

no data available

Potential Health Effects

Inhalation	May be harmful if inhaled. Causes respiratory tract irritation.
Skin	May be harmful if absorbed through skin. Causes skin irritation.
Eyes	Causes eye burns, eye irritation.
Ingestion	Toxic if swallowed. Causes burns.

12. ECOLOGICAL INFORMATION

Elimination information (persistence and degradability)

Refer to component MSDS

Ecotoxicity effects

Refer to component MSDS

Further information on ecology

Refer to component MSDS

13. DISPOSAL CONSIDERATIONS

Product

Observe all federal, state, and local environmental regulations. Contact a licensed professional waste disposal service to dispose of this material.

Contaminated packaging

Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)

No applicable information.

IMDG

No applicable information.

IATA

No applicable information.

15. OTHER INFORMATION

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Hitobiotech, Inc., shall not be held liable for any damage resulting from handling or from contact with the above product. See Terms & Conditions page on our website for additional terms and conditions of sale.

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