



Simple Solutions for Your Research

Hito NeuronMyelinStain™ PreKit

[Catalog Number: HTKNS1225NH]

An easy use staining system for the neurons and Myelin
Fibers Staining on frozen and paraffin sections

User Manual And Material Safety Data Sheet

FOR IN VITRO RESEARCH USE ONLY

Hitobiotec Corp.

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I. Introduction

Myelin is a dielectric (electrically insulating) material that forms a layer, the myelin sheath, usually around only the axon of a neuron. It is essential for the proper functioning of the nervous system¹.

Demyelination is the loss of the myelin sheath insulating the nerves, and is the hallmark of some neurodegenerative autoimmune diseases, inflammation and injury or as complication of diabetes²⁻⁵. This impairs the conduction of signals in the affected nerves, causing impairment in sensation, movement, and cognition.

To determine the extent of demyelination, the technique which allows localization and visualization of the myelin fibers will be useful.

Hito NeuronMyelinStain™ Kit is designed based on the principle of the traditional methods. This kit has proven to be extremely reliable and sensitive for simultaneously demonstrating morphological details of myelin fibers.

Hito NeuronMyelinStain™ Kit has been tested extensively on the brains and spinal cord from several species of animals and it is a simple solution for your research.

For photo samples, please visit our web site at
www.hitobiotec.com

II. Kit Contents

Store Hito NeuronMyelinStain™ PreKit at room temperature

Kit Contents	Standard Kit
Solution-1 (Stock Solution)	10 ml
Solution-2	250 ml
Solution-3	30 ml
Dropper Bottle	1
Staining jars	3
User Manual and MSDS	1



Note

Before using Hito NeuronMyelinStain™ PreKit, You have to check the following **Required Equipments / Materials** in your lab:

1. Cryostat or Microtome, Light microscope
2. 4% PFA
3. Hito Bouin's Plus Solution
4. Dry ice, Isopentane, O.C.T. Compound (for frozen sections). Paraffin embedding Equipment.
5. Hito Super-Safe Slide and Coverslips
6. Double distilled or deionized water
7. Staining jars for Slides wash
8. Ethanol and Xylene
9. Resinous mounting medium

III. Solution-1 Preparation

Hito NeuronMyelinStain™ PreKit Solution-1 is designed to contain Alcohol that is flammable. However, shipping hazardous materials internationally comes with high cost and long waiting time for customs clearance. This PreKit is designed for customers who can purchase absolute Alcohol in their own countries, and then add to the PreKit (Solution-1 Stock Solution) for fast and safe usage of the kit.

Upon receiving of the Prekit, **Carefully** add 120 ml absolute Alcohol (CAS# 64-17-15, ACS Grade, ≥99.5%, 200 proof, absolute) into the Stock Solution (125 ml bottle), tightly close the bottle, shake vigorously until the chemicals dissolved completely.

Check the box for Ethanol on Solution-1 bottle label so it is clear that the bottle contains flammable material now.

The mixture solution is referred to as Solution-1 in the subsequent sections in this user manual.

IV. 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 20% sucrose solution, store at 4°C. Replace 20% 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 molds on the mesh bottom of the sieve-like basket in a manner that preserves the normal shape of the tissue.
13. Immerse 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 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 intact.

16. Place specimen holder / cryostat chuck on dry ice and place 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 (11-25 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 minute) 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, store at 4°C. Replace Hito Bouin's Plus solution 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 cm³ tissue)
 - 70% ethanol for 2 changes of 2 hourss each
 - 95% ethanol for 2 changes of 1.5 hourss each
 - 100% ethanol for 2 changes of 1 hourss 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 hours 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. Embedding 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 5-11 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 Histo 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 slides box at room temperature.

V. Standard Oven Staining Procedure

(not recommended for frozen sections)

1. Place slides in xylene 2 times, 3 - 5 minutes each.
2. Place slides in 100% ethanol 2 times, 3 minutes each.
3. Place slides in 95% ethanol 2 times, 3 minutes each.
4. Mix 1.5 ml Solution-1 and 10.5 ml 95% ethanol in a 12 ml staining jar (provided in the kit), then place slides in the solution mixture. Close the staining jar and place staining jar in 56°C oven overnight (for frozen sections: 40-45°C). This solution mixture is for one time use only.



Note

Important! For frozen sections, 8 hours may be enough for incubation, not longer than 16 hours.

5. Rinse slides in 95% ethanol for 1-3 seconds (1-3 dips).
6. Place slides in double distilled water.
7. Mix 3 ml Solution-2 and 9 ml double distilled water in a 12 ml staining jar (provided in the kit), then rinse slides in the solution mixture for 5-10 seconds (5-10 dips). This solution mixture can be reused for up to 6 slides.
8. Rinse slides in 70% ethanol for 5-10 seconds (6-10 dips).
9. Rinse slides in double distilled water for 1 minute.
10. Repeat steps 7-9 until there is a sharp contrast between the blue of the white-matter and the colorless gray-matter.



Note

This differentiation should be carefully performed, because myelin fiber staining will fade rapidly. Check under the microscope and if over differentiated, return to step 4.

11. Add a drop or two of Solution-3 directly next to tissue and ensure full coverage of the tissue by tapping the slide, wait for 5-30 seconds.
12. Rinse slides in double distilled water for 15 seconds.
13. Rinse slides in double distilled water 2 times, 1 minute each.
14. Remove excess liquid from the slide with a paper towel.
15. Dehydrate in 100% ethanol, 4 changes, 2 minutes each or air dry slides (3-5 hours or overnight) at room temperature.
16. Clear in xylene, 2 times, 3 minutes each, and apply coverslip over section using xylene based resinous mounting medium.
17. Allow to dry. The slide can be viewed after drying by brightfield microscopy.

Results

Myelinated fibers: blue

Nerve cells: purple

VI. Microwave Oven Staining Procedure

(Recommended for frozen & Paraffin sections)

1. Place slides in xylene 2 times, 3 - 5 minutes each.
2. Place slides in 100% ethanol 2 times, 3 minutes each.
3. Place slides in 95% ethanol 2 times, 3 minutes each.
4. Mix 1.5 ml Solution-1 and 10.5 ml 95% ethanol in a 12 ml staining jar (provided in the kit), place slides in the solution mixture and heat the staining jar in microwave oven for 3-6 seconds. This solution mixture is for one time use only.



Warning

Important! - Do not close the staining jar, the jar may explode when heated in the microwave oven with the lid closed. Never heat the straining jar in the microwave oven unattended. Use the lowest power setting or use the defrost setting of the microwave oven. Closely watch the surface of the solution mixture, stop the microwave oven immediately if any sign of boiling is observed.

5. Leave the slides in the solution mixture for additional 15 - 20 minutes until desired intensity is achieved.
6. Rinse slides in 95% ethanol for 1-3 seconds (1-3 dips).
7. Place slides in double distilled water.
8. Mix 3 ml Solution-2 and 9 ml double distilled water in a 12 ml staining jar (provided in the kit), then rinse slides in the solution mixture for 5-10 seconds (5-10 dips). This solution mixture can be reused for up to 6 slides.
9. Rinse slides in 70% ethanol for 5-10 seconds (6-10 dips).
10. Rinse slides in double distilled water for 1 minute.
11. Repeat steps 8-10 until there is a sharp contrast between the blue of the white-matter and the colorless gray-matter.



Note

This differentiation should be carefully performed, because myelin fiber staining will fade rapidly. Check under the microscope and if over differentiated, return to step 4.

12. Rinse slides in double distilled water 3 times, 1 minute each.
18. Add a drop or two of Solution-3 directly next to tissue and ensure full coverage of the tissue by tapping the slide, wait for 5-30 seconds.
19. Rinse slides in double distilled water for 15 seconds.
20. Rinse slides in double distilled water 2 times, 1 minute each.
21. Remove excess liquid from the slide with a paper towel.
22. Dehydrate in 100% ethanol, 4 changes, 2 minutes each or air dry slides (3-5 hours or overnight) at room temperature.
23. Clear in xylene, 2 times, 3 minutes each, and apply coverslip over section using xylene based resinous mounting medium. Allow to dry. The slide can be viewed after drying by bright field microscopy.

Results

Myelinated fibers: blue

Nerve cells: purple

VII. References

- 1 Virchow, R. Über das ausgebreitete Vorkommen einer dem Nervenmark analogen Substanz in den tierischen Geweben. *Pathol. Anat* 6, 10 (1854).
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VIII. Material safety data sheet (MSDS)

Date Updated: 11/01/2016
Version 1.5

1. Product and Company Information

Product Name Hito NeuronMyelinStain™ PreKit
Product Number HTKNS1225NH
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 LuxolFastBlueStain™ PreKit	None	No

Ingredient Name	CAS #	SARA 313
WATER	7732-18-5	No
Disulfo copper phthalocyanine amine salt	1328-51-4	No
Lithium Carbonate	554-13-2	Yes
Cresyl Echt Violet	10510-54-0	No

3. Hazards Identification

EMERGENCY OVERVIEW

Target Organ Effect, Toxic by inhalation, Toxic by ingestion, Toxic by skin absorption, Irritant, Carcinogen. Skin sensitiser, Corrosive

HMIS RATING

HEALTH: 1 FLAMMABILITY: 0 REACTIVITY: 0

NFPA RATING

HEALTH: 1 FLAMMABILITY: 0 REACTIVITY: 0

Potential Health Effects

Inhalation Toxic if inhaled. Material is extremely destructive to the tissue of the mucous membranes and upper respiratory tract.

Skin Toxic if absorbed through skin. Causes skin burns.

Eyes Causes eye burns.

Ingestion Toxic if swallowed.

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

Wash off with soap and plenty of water. Consult a physician.

In case of eye contact

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.

Further information

Use water spray to cool unopened containers.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions

Use personal protective equipment. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Remove all sources of ignition. Beware of vapours accumulating to form explosive concentrations. Vapours can accumulate in low areas.

Environmental precautions

Do not let product enter drains.

Methods for cleaning up

Contain spillage, and then collect with non-combustible absorbent material, (e.g. sand, earth, diatomaceous earth, vermiculite) and place in container for disposal according to local / national regulations. Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Handling

Avoid contact with skin and eyes. Avoid inhalation of vapour or mist. Keep away from sources of ignition - No smoking. Take measures to prevent the build up of electrostatic charge.

Storage

Keep container tightly closed in a dry and well-ventilated place. Store in cool place.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

no data available

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.

Materials to avoid

Strong oxidizing agents, flames and sparks.

Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx)

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 Toxic if inhaled. Material is extremely destructive to the tissue of the mucous membranes and upper respiratory tract. Vapours may cause drowsiness and dizziness. .

Skin May be harmful if absorbed through skin. Causes skin irritation.

Eyes Causes eye burns.

Ingestion Toxic if swallowed.

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)

Not dangerous goods

IMDG

Not dangerous goods

IATA

Not dangerous goods

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. Read Terms & Conditions page on our website for additional terms and conditions of sale.

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