



Simple Solution for Your Research

Hito Nerve Myelin Sheath OptimStain™ Kit

[Catalog Number: HTKNS1229]

An easy to use staining system for
nerve myelin sheath 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 substance rich in lipids and proteins that forms a layer, the myelin sheath, around only the axon of a neuron and acts as insulation. To detect the myelin sheath of peripheral nerves, the traditional method is to use osmic acid staining to obtain images for morphometrical assessment. However, osmic acid is extremely volatile and highly poisonous. It can fix corneas through vapor fixation which can lead to blindness. Inhalation of osmic acid can also lead to pulmonary edema and subsequent death, even at low exposure levels.

Hito Nerve Myelin Sheath OptimStain™ Kit provides a safe and better alternative to osmic acid staining. The kit is non-toxic and easy to use with simplified procedures. It delivers stable and improved staining quality, with minimal overstains / background and artifacts when used properly.

Hito Nerve Myelin Sheath OptimStain™ Kit has been tested on the frozen and paraffin transverse sections of peripheral nerves from several species of animals and proven to be sensitive for demonstrating the morphological details of myelin sheath. 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 Nerve Myelin Sheath OptimStain™ Kit

Solution-1, -2, -3 at -20°C and Solution-4, -5 at 4°C

Thaw frozen Solution-1, -2, -3 at 45°C in an oven before use

Kit Contents	Standard Kit
Solution-1	240 ml
Solution-2	120 ml
Solution-3	120 ml
Solution-4	20 ml
Solution-5	125 ml
Solution-6 (Dropper Bottle)	30 ml
Staining Jars	2
User Manual and MSDS	1



Note

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

1. Cryostat or Microtome and light microscope
2. Paraffin embedding equipment (for paraffin sections)
3. Dry ice, isopentane, O.C.T. compound (for frozen sections), ethanol, xylene, 4% PFA (Hito Cat# HTSHS0102), double distilled or deionized water
4. Slide and coverslips
5. Staining jars for slides wash
6. Resinous mounting medium

III. Tissue Preparation

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 Histo 4% PFA, store at 4°C. Replace 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 cm³ tissue)
 - 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 4-8 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 slide.
14. Place the slides with paraffin sections in a 56°C oven for 2 hours (so the wax just starts to melt) to bond the tissue to the glass. Slides can be stored in a slide box at room temperature.

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 20-30% sucrose solution, store at 4°C. Replace sucrose solution after 12 hours, and continue to store at 4°C for 12 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 **-19~-20°C**.



Note

The -19~-20°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 OCT compound on the surface of the specimen holder / chuck. As the OCT compound 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 (**4-20 µm thickness**) on a cryostat with the chamber temperature set at -19~-20°C.
18. Mount the sections on slide.
19. Air dry slides (1-2 hours) at room temperature. Dried sections should be processed as soon as possible but may be stored in a slide box at - 20 C° for one year.

IV. Staining Procedure

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. Place slides in 75% ethanol 2 times, 3 minutes each.
5. Place slides in 50% ethanol 2 times, 3 minutes each.
6. Rinse slides in distilled water 5 times, 1 minute each.
7. Mix 6 ml Solution-1, 3 ml Solution-2, 3 ml Solution-3 and 0.5 ml Solution-4 in a 12 ml staining jar (provided in the kit). This solution mixture is for one time use only.
8. place slides in the solution mixture and incubate the staining jar in 45°C oven for 0.5-2 hours



Note

At this point the slides should be monitored at 15-30 minute intervals under the microscope to determine the extent of labeling.

9. After staining is complete, rinse the slides in double distilled water 5 times, 1 minute each.
10. Place slides in Solution-5 in a 12 ml staining jar (provided in the kit) for 1 minute at room temperature. This 12 ml Solution-5 can be reused for 8-12 slides.
11. Rinse the slides in double distilled water twice, 1 minute each.
12. (Option: Nuclear Counterstaining) From dropping bottle place a few drops of Solution-6 on the section and allow to stand for 1-3 minutes depending on the desired intensity and then rinse slides in double distilled water twice, 1 minute each.
13. Rinse slides in 70% ethanol for 5-10 seconds (6-10 dips).

14. Place the slides in 95% ethanol 1 times, 1 minute each.
15. Place the slides in 100% ethanol 2 times, 3 minutes each.
16. Clear in xylene, 2 times, 3 minutes each. Apply coverslip over sections using xylene based resinous mounting medium, allow to dry. The slides can be viewed after drying by bright field microscopy.

V. 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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- 4 Emard, J. F., Thouez, J. P. & Gauvreau, D. Neurodegenerative diseases and risk factors: a literature review. *Soc Sci Med* 40, 847-858, doi:027795369400138J [pii] (1995).
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VI. Material safety data sheet (MSDS)

Date Updated: 11/01/2013

Version 1.2

1. Product and Company Information

Product Name Hito Nerve Myelin Sheath OptimStain™ Kit
Product Number HTKNS1229
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 Nerve Myelin Sheath OptimStain™ Kit	None	No

Ingredient Name	CAS #	SARA 313
WATER	7732-18-5	No
Sodium phosphate dibasic	7558-79-4	No
Sodium phosphate monobasic	7558-80-7	No
Sodium Acetate	6131-90-4	No
Cresyl Violet	10510-54-0	No
PROPRIETARY COMPONENT(S)	None	No

3. Hazards Identification

EMERGENCY OVERVIEW

May be harmful if inhaled. May cause respiratory tract irritation, May cause an allergic skin reaction, May cause eye irritation, May be harmful if swallowed

HMIS RATING

HEALTH: 1 FLAMMABILITY: 0 REACTIVITY: 0

NFPA RATING

HEALTH: 1 FLAMMABILITY: 0 REACTIVITY: 0

Potential Health Effects

Inhalation May be harmful if inhaled. May cause respiratory tract irritation

Skin Toxic if absorbed through skin. Causes skin burns.

Eyes Causes eye burns.

Ingestion May be harmful 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.

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 May be harmful if inhaled. May cause respiratory tract irritation

Skin May cause an allergic skin reaction

Eyes Causes eye burns.

Ingestion May be harmful 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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