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Simple Solutions for Your Research

# Hito NeuronMyelinStain™ Kit

**[Catalog Number: HTKNS1225]**

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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# Index

# 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 system[1].

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 diabetes[2-5]. 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™ Kit at room temperature**

| Kit Contents | Standard Kit | Small Kit |
|---|---|---|
| Solution-1 | 125 ml | 50 ml |
| Solution-2 | 250 ml | 100 ml |
| Solution-3 | 30 ml | 15 ml |
| Dropper Bottle | 1 | 1 |
| Staining jars | 3 | 2 |
| User Manual and MSDS | 1 | 1 |

### Note

Before using Hito NeuronMyelinStain™ Kit, 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. 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 at - 20 C° 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 $cm^3$ 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 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 slides box at room temperature.

## IV. 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

## V. 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

# VI. References


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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).

5 Andjelkovic, A. V. & Pachter, J. S. Central nervous system endothelium in neuroinflammatory, neuroinfectious, and neurodegenerative disease. J Neurosci Res 51, 423-430, doi:10.1002/(SICI)1097-4547(19980215)51:4<423::AID-JNR2>3.0.CO;2-E [pii] (1998).

# VII. Material safety data sheet (MSDS)

Date Updated: 12/01/2016
Version 1.6

## 1. Product and Company Information

| | |
|---|---|
| **Product Name** | Hito NeuronMyelinStain™ Kit |
| **Product Number** | HTKNS1225 |
| **Brand** | Hitobiotec |
| **Company Address** | Hitobiotec Corp.<br>P.O.Box 7528<br>Kingsport, TN 37664<br>USA |
| **Technical Phone:** | 423-520-6880 |
| **Emergency Phone:** | 423-520-6880 |

## 2. Composition and Information on Ingredient

| **Substance Name** | **CAS #** | **SARA 313** |
|---|---|---|
| Hito LuxolFastBlueStain™ Kit | 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 |
| Ethyl Alcohol (ETHANOL) | 64-17-5 | Yes |
| Cresyl Echt Violet | 10510-54-0 | No |

## 3. Hazards Identification

**EMERGENCY OVERVIEW**

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

**HMIS RATING**

HEALTH: 1 FLAMMABILITY: 3 REACTIVITY: 0

**NFPA RATING**

HEALTH: 1 FLAMMABILITY: 3 REACTIVITY: 0

**Potential Health Effects**

| | |
|---|---|
| **I**nhalation | 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)**

UN-Number: 3316 Class: 9 Packing Group: III
Proper shipping name: Chemical kits

**IMDG**

UN-Number: 3316 Class: 9 Packing Group: III EMS-No: F-A, S-P
Proper shipping name: Chemical kits

**IATA**

UN-Number: 3316 Class: 9 Packing Group: III
Proper shipping name: Chemical kits

## 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.

## Notes

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