HITO
Biotec in situ detection

Simple Solution for Your Research

Hito Luxol Fast Blue - PAS OptimStain™ PreKit

[Catalog Number: HTKNS1228NH]

An easy to use staining system for neurons and
myelinated fibers 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

Hito Luxol Fast Blue - PAS OptimStain™ Kit is designed based on Luxol fast blue and Periodic acid-Schiff (PAS) method. This kit is made in a ready-to-use format and offers high quality, rapid staining (can be finished in one hour) of myelin/myelinated axons on paraffin sections or frozen sections. It has been proven to be extremely reliable and sensitive to demonstrate the morphological details of myelin fibers.

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. It is essential for the proper functioning of the nervous system. Demyelination is the loss of the myelin sheath insulating the nerves. This impairs the conduction of signals in the affected nerves, causing impairment in sensation, movement, and cognition. Currently no cure exists for demyelinating diseases and myelin repair is an active research field. Hito Luxol Fast Blue - PAS OptimStain™ Kit allows sensitive localization and visualization of the myelin fibers. PAS counterstain provides color contrasts which show morphological features that are particularly significant in the tissue, thus offers a fast and reliable way to determine the extent of demyelination.

Hito Luxol Fast Blue - PAS OptimStain™ Kit has been tested extensively on the brains and spinal cords 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 Luxol Fast Blue - PAS OptimStain™ PreKit at room temperature

Kit Contents	Standard Kit
Solution-1 (Stock Solution)	10 ml
Solution-2	250 ml
Solution-3	125 ml
Solution-4	125 ml
Solution-5	30 ml
Solution-6	125 ml
Dropper Bottle	1
Staining Jars	6
User Manual and MSDS	1

Note

Before using Hito LFB - PAS OptimStain™ PreKit, 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. Hito Bouin's Plus Solution (Cat# HTSHS0104)
4. Dry ice, isopentane, O.C.T. compound (for frozen sections), ethanol, xylene, 4% PFA (Hito Cat# HTSHS0102), double distilled or deionized water
5. Slide and coverslips
6. Staining jars for slides wash
7. Resinous mounting medium

III. Solution-1 Preparation

Hito Luxol Fast Blue - PAS OptimStain™ Kit 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 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 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 (56-58ºC), 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 3-7 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% 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 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 (5-11 µm thickness, **thinner sections have better results**) on a cryostat with the chamber temperature set at -17°C.
18. Mount the sections on 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 - 20 C° for one year.

V. Standard Oven Staining Procedure

(Recommended for 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), then place slides in the solution mixture. Tightly 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 4 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. Rinse slides in double distilled water 3 times, 1 minute each.
12. Fill 12ml Solution-3 in a 12 ml staining jar which is provided in the kit. Place slides in Solution-3 for 5 minutes at room temperature. This 12 ml Solution-3 can be reused for up to 20 slides.
13. Rinse the slides in double distilled water 4 times, 15 seconds each.
14. Fill 12 ml Solution-4 in a 12 ml staining jar which is provided in the kit. Place slides in Solution-4 for 5-15 minutes at room temperature. This 12 ml Solution-4 can be reused for up to 20 slides.
15. Rinse the slides in double distilled water 4 times, 3 minutes each.
16. Nuclear counter staining (optional step)

    Using the dropping bottle (provided in the kit), place a few drops of Solution-5 on the section to fully cover the sections, wait for 15-30 seconds.

    Rinse slides in double distilled water for 1 minute.
17. Mix 6 ml Solution-6 and 6 ml double distilled water in a 12 ml staining jar (provided in the kit), then rinse slides in the solution mixture for 1-3 minutes. This solution mixture can be reused for up to 12 slides.
18. Rinse slides in double distilled water for 1 minute.
19. Dehydrate in 100% ethanol, 4 changes, 2 minutes each.
20. 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.

VI. Microwave Oven Staining Procedure

(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 10 ml staining jar (provided in the kit), place slides in the solution mixture and heat the staining jar in microwave oven for 3-15 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 4 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.
13. Fill 12 ml Solution-3 in a 12 ml staining jar which is provided in the kit. Place slides in Solution-3 for 5 minutes at room temperature. This 12 ml Solution-3 can be reused for up to 20 slides.
14. Rinse the slides in double distilled water 4 times, 15 seconds each.
15. Fill 12 ml Solution-4 in a 12 ml staining jar which is provided in the kit. Place slides in Solution-4 for 5-15 minutes at room temperature. This 12 ml Solution-4 can be reused for up to 20 slides.
16. Rinse the slides in double distilled water 4 times, 3 minutes each.
17. Nuclear counter staining (optional step)

    Using the dropping bottle (provided in the kit), place a few drops of Solution-5 on the section to fully cover the sections, wait for 15-30 seconds.

    Rinse slides in double distilled water for 1 minute.
18. Mix 6 ml Solution-6 and 6 ml double distilled water in a 12 ml staining jar (provided in the kit), then rinse slides in the solution mixture for 1-3 minutes. This solution mixture can be reused for up to 12 slides.
19. Rinse slides in double distilled water for 1 minute.
20. Dehydrate in 100% ethanol, 4 changes, 2 minutes each.
21. 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.

VII. References

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VIII. Material safety data sheet (MSDS)

Date Updated: 11/01/2014
Version 1.4

1. Product and Company Information

Product Name	Hito Luxol Fast Blue - PAS OptimStain™ PreKit
Product Number	HTKNS1228NH
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 Luxol Fast Blue - PAS OptimStain™ PreKit	None	No

Ingredient Name	**CAS #**	**SARA 313**
WATER	7732-18-5	No
Luxol fast blue	1328-51-4	No
Lithium Carbonate	554-13-2	Yes
Periodic acid	10450-60-9	No
Basic Fuchsin	632-99-5	No
Hematoxylin	517-28-2	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: 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. See Terms & Conditions page on our website for additional terms and conditions of sale.

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