



Simple Solution for Your Research

Hito Luxol Fast Blue GMA OptimStain™ Kit

[Catalog Number: HTKNS1230]

An easy to use staining system for myelinated fibers on glycolmethacrylate embedding (GMA) 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 Luxol Fast Blue GMA OptimStain™ Kit provides a safe and better alternative to osmic acid staining. The kit is designed based on Luxol fast blue method, it is non-toxic and made in a ready-to-use format. This kit offers high quality, rapid staining of myelin sheath of peripheral nerves on the glycolmethacrylate embedded (GMA) sections, proven to be reliable and sensitive to demonstrate the morphological details of myelin sheath of peripheral nerves. It is a simple solution for your research.

II. Kit Contents

Store Hito Luxol Fast Blue GMA OptimStain™ Kit

at room temperature

Kit Contents

Solution-1	50 ml
Solution-2	50 ml
Solution-3	30 ml
Dropper Bottle	1
Staining Jars	3
User Manual and MSDS	1



Note

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

1. Glycolmethacrylate embedded (GMA) sections on slides
2. ethanol, xylene, double distilled or deionized water
3. coverslips
4. Staining jars for slides wash
5. Resinous mounting medium

III. Staining Procedure

1. Preheat oven to 65°C.
2. Place slides in 95% ethanol for 1 minute.
3. Mix 2.5 ml Solution-1 and 9.5 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 5 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.

4. Then heat the staining jar in microwave oven additional 6 times, 2 seconds each.
5. Close the staining jar and place staining jar in 65°C oven for 20 minutes.
6. Remove the staining jar from the oven and leave the slides in the solution mixture for additional 15 - 20 minutes to cool down.
7. Rinse slides in 70% ethanol for 1-3 seconds (1-3 dips).
8. Place slides in double distilled water.
9. Mix 2.5 ml Solution-2 and 9.5 ml double distilled water in a 12 ml staining jar (provided in the kit), then rinse slides in the solution mixture for 1-3 seconds (1-3 dips). This solution mixture can be reused for up to 4 slides.
10. Rinse slides in double distilled water for 1 minute.
11. Rinse slides in 70% ethanol for 1-3 seconds (1-3 dips).
12. Rinse slides in double distilled water for 1 minute.

13. Repeat steps 8-12 until there is a sharp contrast between the blue myelin sheath and other tissue.



Note

This differentiation should be performed carefully because myelin fiber staining will fade rapidly. Check under the microscope after step 12 each time. Return to step 1 if over differentiated.

14. Place slides in double distilled water.
15. Nuclear counter staining (optional step)
16. Using the dropping bottle containing Solution-3, place a few drops of Solution-3 on the section to fully cover the section, wait for 5-15 minutes.
17. Rinse slides in double distilled water 3 times, 3 minutes each.
18. Air dry tissue slides (1-3 hours or overnight) at room temperature.
19. 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.

IV. 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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- 3 Reske-Nielsen, E. & Lundbaek, K. Pathological changes in the central and peripheral nervous system of young long-term diabetics. II. The spinal cord and peripheral nerves. *Diabetologia* 4, 34-43 (1968).
- 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).

V. Material safety data sheet (MSDS)

Date Updated: 12/29/2015

Version 1.0

1. Product and Company Information

Product Name Hito Luxol Fast Blue GMA OptimStain™ Kit
Product Number HTKNS1230
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 GMA OptimStain™ Kit	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
Ethyl Alcohol (ETHANOL)	64-17-5	Yes
PROPRIETARY COMPONENT(S)	None	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

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 foam, 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. Hitobitech, 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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