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Simple Solution for Your Research

Hito CryoMyelinStain™ Nissl Kit

[Catalog Number: HTKNS1231]

An easy to use staining system for
myelinated fibers
on frozen (mounted or floating) sections

User Manual
and
Material Safety Data Sheet

FOR IN VITRO RESEARCH USE ONLY

Hitobiotec Corp.

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I. Introduction

Hito CryoMyelinStain™ Nissl Kit is made in a ready-to-use format and offers high quality, rapid staining of myelin/ myelinated axons and nuclear counterstaining on frozen sections. This kit has many advantages compared to the traditional Luxol fast blue staining method which is time-consuming, requires usage of 40-56°C incubator, and usually has low yields of stained myelin fibers and unreliable results. In addition, the long time high temperature incubation process of the frozen sections may cause sections felling off from the slides. Hito CryoMyelinStain Nissl Kit offers a simple solution to these problems. The procedures are simplified and the processing time is greatly reduced. Users can use mounted sections or floating sections at room temperature. This kit delivers stable and improved staining quality. It has been proven to be extremely reliable and sensitive for demonstrating the morphological details of myelin fibers.

Myelin is essential for the proper functioning of the nervous system[1]. Demyelination impairs the conduction of signals in the affected nerves, causing impairment in sensation, movement, and cognition[2-5]. Currently no cure exists for demyelinating diseases and myelin repair is an active research field. Hito CryoMyelinStain™ Nissl Kit allows sensitive localization and visualization of the myelin fibers, thus offers a fast and reliable way to determine the extent of demyelination.

Hito CryoMyelinStain™ Nissl 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 www.hitobiotec.com

II. Kit Contents

Store Hito CryoMyelinStain™ Nissl Kit at 4°C in the dark

Kit Contents	
Solution-1	50 ml
Solution-2	3 ml
Solution-3	12 ml
Solution-4	250 ml
Solution-5	25 ml
Solution-6	30 ml
Hito Aqua Barrier PAP Pen (HTHS0110)	1
Dropper Bottle	2
Fine Tip Natural Hair Brush	1
Glass Specimen Transfer Tool	1
Staining Jar	3
User Manual and MSDS	1

Note

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

1. Cryostat and light microscope
2. Dry ice, isopentane, O.C.T. compound, ethanol, xylene, 4% PFA (recommend Hito Buffered 4% Paraformaldehyde Solution Cat# HTSHS0102), double distilled or deionized water
3. Gelatin coated slides and coverslips
4. Staining jars for slides wash
5. Resinous mounting medium

III. Tissue Preparation

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 in 0.1M PB 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 (in 0.1M PB), 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 30% sucrose (in 0.1M PB) solution, store at 4°C. Replace 30% 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 of **30-60** µm thickness, on a cryostat with the chamber temperature set at -17°C.

18. Mount the sections on slides.
19. Air dry the slides 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.
20. For **floating sections**, collect the sections in **Solution-1** (10 ml Solution-1 for 50 mouse brain / 25 rat brain sections) and store at -20°C until staining is performed (sections may be stored in Solution-1 at -20°C for one year).

IV. Stain Solution Preparation

Simply add Solution-2 into Solution-3 and mix.

Once mixed, the mixed Solution-2 and -3 should be stored in the dark at 4°C and is good for up to 3 months.

V. Staining Procedure

For mounted sections:

1. Place slides in double distilled water 3 times, 5 minutes each.
2. Remove excess liquid from the slides with a paper towel.
3. Draw a loose circle with Hito Aqua Barrier PAP Pen (provided in the kit) around the rehydrated sections. After the Hito Aqua Barrier PAP Pen circle dries, using the dropping bottle, add a few drops of the mixed stain solution on the sections and incubate slides in a dark wet box (humidified chamber) for 0.5 - 4 hours at 37°C.

Note

At this point the slides should be monitored at 15-30 minute intervals under the microscope to determine the extent of labeling. Staining is complete when the finest myelinated fibers are stained dark red. The appearance of a lavender background color indicates over staining and the process should be stopped. The exact staining time will vary depending on section thickness, fixing conditions, type of tissues, and age of the stain solution.

4. After staining is complete, rinse the slides in double distilled water for 3-6 seconds (3-6 dips).
5. Place the slides in Solution-4 (10-12 ml for 4-6 slides) for 3 minutes.
6. Place the slides in double distilled water 2 times, 1 minute each.
7. Place the slides in 75% ethanol 2 times, 3 minutes each.
8. Place the slides in 95% ethanol 2 times, 3 minutes each.

9. Place the slides in 100% ethanol 2 times, 3 minutes each.
10. Place the slides in xylene, 2 times, 3 minutes each.
11. Place the slides in 100% ethanol 2 times, 3 minutes each.
12. Place the slides in 95% ethanol 2 times, 3 minutes each.
13. Place the slides in 75% ethanol for 3 minutes.
14. Place the slides in double distilled water 3 times, 1 minute each.
15. Remove excess liquid from the slides with a paper towel.
16. Using the dropping bottle, add a few drops of the Solution-6 on the sections and incubate slides for 1 - 5 minutes at room temperature.
17. Rinse slides in distilled water for 1 minute.
18. Place the slides in 100% ethanol 2 times, 3 minutes each.
19. Place the slides 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.

For floating sections:

1. Wash sections in double distilled water for 3 times, 5 minutes each.
2. Place sections in the stain solution mixture (solution-2 and -3) for 0.5-4 hours, in a covered glass / plastic tube or vial at 37°C and in the dark (1 ml stain solution mixture for 15 mouse brain / 10 rat brain sections).

Note

At this point the sections should be monitored at 15-30 minute intervals to determine the extent of labeling. Staining is complete when the finest myelinated fibers are stained dark red. The appearance of a lavender background color indicates over staining and the process should be stopped. The exact staining time will vary depending on section thickness, fixing conditions, type of tissues, and age of the stain solution.

3. After staining is complete, rinse the sections in double distilled water for 3 times, 1 minute each.
4. Rinse the sections in Solution-4 (5 ml for 25 mouse brain / 15 rat brain sections) for 2-3 minutes.
5. Rinse the sections in double distilled water for 3 times, 1 minute each.
6. Wash the sections in 37°C pre-warmed Solution-5 for 1 minute (1ml Solution-5 for 10 mouse brain / 5 rat brain sections).
7. Place the sections in double distilled water using a Glass Specimen Transfer Tool. Float individual section onto the slides using a Fine Tip Natural Hair Brush (provided in the kit).
8. Air dry tissue slides for 3-5 hours or overnight at room temperature and in the dark.

9. Place the slides in xylene, 2 times, 3 minutes each.
10. Place the slides in 100% ethanol 2 times, 3 minutes each.
11. Place the slides in 95% ethanol 2 times, 3 minutes each.
12. Place the slides in 75% ethanol for 3 minutes.
13. Place the slides in double distilled water 3 times, 1 minute each.
14. Remove excess liquid from the slides with a paper towel.
15. Using the dropping bottle, add a few drops of the Solution-6 on the sections and incubate slides for 1 - 5 minutes at room temperature.
16. Rinse slides in distilled water for 1 minute.
17. Place the slides in 100% ethanol 2 times, 3 minutes each.
18. Place the slides 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

Results

Myelinated fiber: dark red

Cell nuclear: blue

VI. References

1 Virchow, R. Über das ausgebreitete Vorkommen einer dem Nervenmark analogen Substanz in den tierischen Geweben. Pathol. Anat 6, 10 (1854).

2 Connolly, R. C. Delayed spinal cord lesions following injury. Riv Patol Nerv Ment 86, 225-229 (1965).

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).

VII. Material safety data sheet (MSDS)

Date Updated: 12/05/2018
Version 1.6

1. Product and Company Information

Product Name	Hito CryoMyelinStain™ Nissl Kit
Product Number	HTKNS1231
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 CryoMyelinStain™ Nissl 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 Thiosulfate	10102-17-7	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: 0 FLAMMABILITY: 0 REACTIVITY: 0

NFPA RATING

HEALTH: 0 FLAMMABILITY: 0 REACTIVITY: 0

Potential Health Effects

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation
Skin	May cause an allergic skin reaction
Eyes	May cause eye irritation
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	May cause eye irritation
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.

Notes

Notes

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