HITO
Biotec in situ detection

Simple Solution for Your Research

Hito Perls' Iron OptimStain™ Kit

[Catalog Number: HTKMS1002]

An easy to use staining system for the morphological characterization of Iron in paraffin tissue sections

User Manual
And
Material Safety Data Sheet

FOR IN VITRO RESEARCH USE ONLY

Hitobiotec Corp.

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Index

I. Introduction

The Perl's stain is the most commonly used method to detect the presence of iron in biopsy specimens. It depends on the Prussian blue reaction. The original stain formula, known historically (1867) as "Perls' Prussian blue" after its inventor, German pathologist Max Perls (1843-1881), used separate solutions of potassium ferrocyanide and acid to stain tissue, the tissue is first treated with dilute hydrochloric acid to release ferric ions from binding proteins. The freed ions then react with potassium ferrocyanide to form insoluble Prussian blue dye (a complex hydrated ferric ferrocyanide substance) in situ.

Hito Perls' Iron OptimStain™ Kit designed based on the Perl's stain, this kit makes dramatic improvement of the Perls' Prussian blue technique. The procedures are simplified and the processing time is reduced. This kit delivers stable and improved staining quality. 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 Perls' Iron OptimStain™ Kit

at room temperature

Kit Contents	
Solution-1A	125 ml
Solution-1B	125 ml
Solution-2	125 ml
Solution-3 (counterstain)	30 ml
Dropper Bottle (30 ml Solution-3)	1
Staining Jars	2
User Manual and MSDS	1

Note

Before using Hito Perls' Iron OptimStain™ Kit, please make sure you have the following Required Equipment / Materials in your lab (not included in the kit)::

1. Microtome and light microscope
2. Paraffin embedding equipment (for paraffin sections)
3. Ethanol, xylene, 4% PFA (Hito Cat# HTSHS0102)
4. Double distilled or deionized water
5. Slide and coverslips
6. Staining jars for slides wash
7. 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 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 solution, store at 4°C. Replace 4% PFA solution after 24 hours, and continue to store at 4°C for 24-48 hours.

Note

Avoid using iron containing materials and jars while fixing as these may contaminate the tissue. Avoid using acid containing fixatives (e.g., Picric acid, Acetic acid), The acid containing fixatives may remove the iron deposits.

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 (56-58°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 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.

IV. Staining Procedure

1. Deparaffin and rehydrate the sections by placing the slides in xylene, 100% ethanol, 95% ethanol, 75% ethanol, 50% ethanol and then in double distilled water with 2 changes in each solvent, and 5-7 minutes during each change.
2. Mix 3 ml Solution-1A, 3 ml Solution-1B and 6 ml distilled water in a 12 ml staining jar (provided in the kit), then place slides in the solution mixture, close and place the staining jar at room temperature for 30-60 minutes. This solution mixture is for one time use only.
5. Rinse slides in double distilled water for two times, 3 minutes each.
6. Mix 3 ml Solution-2 and 9 ml double distilled water in a 12 ml staining jar (provided in the kit), then place slides in the solution mixture for 30 seconds. This solution mixture can be reused for up to 8-10 slides.
7. Rinse slides in double distilled water for 1 minute.
8. Using the dropping bottle (provided in the kit), place a few drops of Solution-3 on the section to fully cover the sections. Allow to stand for 30-45 seconds.
9. Rinse slides in double distilled water for 1 minute.
10. Rinse slides in 95% ethanol for 5-10 seconds (5-10 dips).
11. Repeat step 10 until there is a sharp contrast between the nucleus and cytoplasm.

Note

This differentiation should be carefully performed, because nuclear staining will fade rapidly. Check under the microscope and if over differentiated, return to step 7.

12. Dehydrate in 100% ethanol, 3 changes, 2 minutes each.
13. 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.

V. Material safety data sheet (MSDS)

Date Updated: 11/01/2016
Version 1.2

1. Product and Company Information

Product Name	Hito Perls' Iron OptimStain™ Kit
Product Number	HTKMS1002
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 Perls' Iron OptimStain™ Kit	None	No

Ingredient Name	**CAS #**	**SARA 313**
WATER	7732-18-5	No
Potassium ferrocyanide solution	14459-95-1	No
PROPRIETARY COMPONENT(S)	None	No

3. Hazards Identification

EMERGENCY OVERVIEW

Causes severe skin burns and eye damage.

HMIS RATING

HEALTH: 1 FLAMMABILITY: 0 REACTIVITY: 0

NFPA RATING

HEALTH: 1 FLAMMABILITY: 0 REACTIVITY: 0

Potential Health Effects

Inhalation	Toxic if inhaled.
Skin	Causes skin burns.
Eyes	Causes eye damage.
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. Consult a physician.

In case of skin contact

Take off contaminated clothing and shoes immediately. 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.Continue rinsing eyes during transport to hospital.

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

Soak up with inert absorbent material and dispose of as hazardous waste. Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Handling

Avoid contact with skin and eyes. Avoid inhalation of vapour or mist. Use explosion-proof equipment.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. Keep container tightly closed in a dry and wellventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage. Never allow product to get in contact with water during storage. Do not store near acids.

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. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Eye protection

Safety glasses with side-shields conforming to EN166

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

Refer to component MSDS

Hazardous decomposition products

Refer to component MSDS

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.
Skin	Causes skin burns.
Eyes	Causes eye damage.
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.

Notes

Notes

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