HITO
Biotec in situ detection

Simple Solution for Your Research

Hito Verhoeff OptimStain™ Kit

[Catalog Number: HTKCS0101]

An easy to use staining system for the collagen and elastic fiber 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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I. Introduction

Hito Verhoeff OptimStain™ Kit is designed based on the Verheoff and van Gieson staining method with improved and simplified procedures. This kit can be used for simultaneously demonstrating the morphological details of collagen and elastic fibers.

One example application of this kit is to determine the extent of atherosclerosis. The atherosclerotic plaque contains lipids, inflammatory cells, smooth muscle cells, connective tissue (eg, collagen, glycosaminoglycans, elastic fibers), thrombi, and calcium deposits. Fibrillar collagen is a critical component of atherosclerotic lesions. Uncontrolled collagen accumulation leads to arterial stenosis, while excessive collagen breakdown combined with inadequate synthesis weakens plaques thereby making them prone to rupture[1-10]. With its special design, Hito Verhoeff OptimStain™ Kit will allow localization and visualization of the collagen and damage of elastic structure, therefore, enables a good understanding of the extent of atherosclerosis. This kit can also be used to demonstrate changes in elastic tissues in case of emphysema and other vascular diseases.

Hito Verhoeff OptimStain™ Kit is made in a ready-to-use format and provides high quality, reliable and sensitive staining of collagen and elastic fibers.

Hito Verhoeff OptimStain™ Kit has been tested extensively on the hearts and arteries 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 Verhoeff OptimStain™ Kit at room temperature

Solution-1A	125 ml
Solution-1B	55 ml
Solution-1C	55 ml
Solution-2	250 ml
Solution-3	250 ml
Solution-4	250 ml
Solution-5	250 ml
Staining jars	6
User Manual and MSDS	1

Note

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

1. Cryostat or Microtome, Light microscope
2. Paraffin embedding equipment (or paraffin sections)
3. Hito Bouin's Plus Solution (Cat# HTSH0102 , for paraffin sections preparation)
4. Dry ice, isopentane, O.C.T. compound (for frozen sections), 4% PFA (Cat# HTSH0101), ethanol, xylene, double distilled or deionized water
5. Slide and coverslips
6. Staining jars for slides wash
7. Resinous mounting medium

III. Tissue Preparation

For Heart Frozen Section (NON-FIXED)

1. 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.
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. **Do not** perfuse with fixative. Cut all the fat and tissue surrounding the heart, including the pulmonary artery, and veins.
5. Flush the heart through the right ventricle, left atrium, and left ventricle using a 10 ml syringe containing a total of 3 ml of PBS to clean out residual blood.
6. Remove the heart as soon as possible but this process must be carried out very carefully to avoid damage of the tissue.
7. Locate the left and right atria. Using a sharp razor blade, cut the bottom half of the heart off in a plane parallel to the atria.

Note

It is essential that the gross cut is parallel to the atria so that a cross section of all three aortic valves is in the same geometric plane. Discard lower half of the heart.

8. Rinse tissue briefly in double distilled water for 1-2 sec. to remove blood from the surface.

9. Place the trimmed heart in OCT compound in a base mold on the mesh bottom of the sieve-like basket.
10. 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.

11. Wrap the dried, frozen tissue block in aluminum foil and store at -70°C until sectioning is performed.
12. 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 maintain integrity.

13. 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.
14. Slowly cut the tissue into sections (6-15 µm thickness) on a cryostat with the chamber temperature set at -17°C.
15. Mount the sections direct on the Hito Super-Safe Slide
16. Air dry slides (90 minutes) at room temperature. Dried sections should be processed as soon as possible but may be stored in a slide box at -70°C for one year.

For Other Frozen Tissue Section (FIXED)

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 30% sucrose 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 maintain 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 (6-15 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 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.

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 45ºC. Use fresh deionized water. Insert the block into the microtome chuck. Set the dial to cut 4-10 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 slide box at room temperature.

IV. Staining Procedure

Staining

(For Fixed, non-Fixed frozen sections and paraffin sections)

1. Place slides in xylene two times, 5 minutes each.
2. Place slides in 100% ethanol two times, 3 minutes each.
3. Place slides in 95% ethanol two times, 3 minutes each.
4. Place slides in 75% ethanol for 3 minutes.
5. Place slides in 50% ethanol for 3 minutes.
6. Rinse slides in double distilled water for 3 minutes.
7. Mix 6.2 ml Solution-1A, 2.5 ml Solution-1B and 2.5 ml Solution-1C in a 12 ml staining jar then place slides in the solution mixture and wait for 10-15 minutes at room temperature. (This solution mixture must be freshly prepared and must be used within 2 hours and 12 ml solution mixture can be used for 8-10 slides).
8. Place slides in double distilled water two times, 30 seconds each, with renewed double distilled water.
9. Dip slides in Solution-2 to differentiate, 12 ml solution-2 can be used for 8 slides.

Note

This differentiation should be carefully performed, because elastic fiber staining will fade rapidly. Check under microscope and if over differentiated, return to Step 7.

10. Rinse slides in double distilled water for 1 minute.
11. Rinse slides in Solution-3 for 1 minute, 12 ml solution-3 can be used for 8 slides.
12. Rinse slides in double distilled water for 15 seconds.
13. Place slides in Solution-4 for 3 minutes, 12 ml solution-4 can be used for 8 slides.

14. Rinse slides in double distilled water for 1 minute.
15. Place slides in Solution-5 for 1 minute, 12 ml solution-5 can be used for 8 slides.
16. Dip slides in 95% Alcohol to differentiate (Dip for about four times).

Note

This differentiation should be carefully performed, because yellow tissue staining will fade rapidly. Check under microscope and if over differentiated, return to Step 15.

17. Dehydrate slides in 100% ethanol, two times, 3 minutes each.
18. Clear in xylene, two times, 3 minutes each, and apply coverslip over sections using xylene based resinous mounting medium.
19. Allow to dry. The slide can be viewed after drying by bright field microscopy.

Results

Elastic Fibers: *Black*

Collagen: *Red*

Nuclei: *Gray*

Other tissue elements: *Yellow*

V. References

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7 Weyand, C. M. & Goronzy, J. J. Pathogenic mechanisms in giant cell arteritis. Cleve Clin J Med 69 Suppl 2, SII28-32 (2002).

8 Lemaitre, V., Soloway, P. D. & D'Armiento, J. Increased medial degradation with pseudo-aneurysm formation in apolipoprotein E-knockout mice deficient in tissue inhibitor of metalloproteinases-1. Circulation 107, 333-338 (2003).

9 Seyama, Y. & Wachi, H. Atherosclerosis and matrix dystrophy. J Atheroscler Thromb 11, 236-245, doi:JST.JSTAGE/jat/11.236 [pii] (2004).

10 Fabrizio Rodella, L. et al. Atherosclerosis and the protective role played by different proteins in apolipoprotein E-deficient mice. Acta Histochem 109, 45-51, doi:S0065-1281(06)00090-0 [pii] 10.1016/j.acthis.2006.08.002 (2007).

VI. Material safety data sheet (MSDS)

Date Updated: 11/07/2014
Version 2.3

1. Product and Company Information

Product Name	Hito Verhoeff OptimStain™ Kit
Product Number	HTKCS0101
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 Verhoeff OptimStain™ Kit	None	No

Ingredient Name	**CAS #**	**SARA 313**
WATER	7732-18-5	No
PROPRIETARY COMPONENT(S)	None	No
Acid Fuchsin	3244-88-0	No
Picric Acid	88-89-1	Yes
Ethyl Alcohol (ETHANOL)	64-17-5	Yes
Haematoxylin	517-28-2	No
Ferric Chloride	10025-77-1	No
Potassium Iodine	7681-11-0	No
Sodium Thiosulfate	7681-57-4	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: 2 FLAMMABILITY: 3 REACTIVITY: 0

NFPA RATING

HEALTH: 2 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 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, Heat, 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. See Terms & Conditions page on our website for additional terms and conditions of sale.

Notes

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