Simple Solution for Your Research

Hito Fat-Elastic Fiber OptimStain™ Kit

[Catalog Number: HTKLE0101]

An easy to use staining system for the
lipid and elastic fiber staining on frozen sections

User Manual
And
Material Safety Data Sheet

FOR IN VITRO RESEARCH USE ONLY

Hitobiotec Inc.

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I. Introduction

Hito Fat-Elastic Fiber OptimStain™ Kit is designed based on the Oil Red O staining method and the Verheoff staining method with improved and simplified procedures. This kit can be used for simultaneously demonstrating the morphological details of lipids and elastic fibers. For example, to determine the extent of atherosclerosis, this kit will allow localization and visualization of both the lipids (e.g., intracellular and extracellular cholesterol and phospholipids) and any damage to the connective tissue (eg, elastic fibers) in the atherosclerotic plaque [1-5]. This kit can also be used to demonstrate changes in elastic tissues in case of emphysema and other vascular diseases.

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

Hito Fat-Elastic Fiber 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 Fat-Elastic Fiber OptimStain™ Kit

at room temperature

Solution-1	450 ml
Solution-2	125 ml
Solution-3A	125 ml
Solution-3B	55 ml
Solution-3C	55 ml
Solution-4	250 ml
Solution-5	250 ml
Solution-6	250 ml
Staining jars	7
Hito AquaMount™ Mounting Medium	20 ml
User Manual and MSDS	1

Note

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

1. Cryostat (be able to cut 6- to 15-μm thick sections)
2. Dry ice, isopentane, O.C.T. compound, double distilled or deionized water
3. Slide and coverslips
4. Staining jars for slides wash
5. Light microscope

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 molds 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 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 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.

IV. Staining Procedure

Post-Fixation

(For non-Fixed frozen tissue section only)

1. Place slides in 4% PFA for 5 minutes.
2. Rinse slides in distilled water three times, 1 minute each. Distilled water should be renewed frequently.
3. Air dry slides at room temperature. Dried sections should be stained as soon as possible.

Note

The post-fixation is only for non-fixed frozen tissue sections, unnecessary for the fixed tissue sections.

Staining

(For Fixed and post-Fixed frozen sections)

1. Rinse slides in Solution-1 for 1 minute. Solution-1 can be reused for Step 3.
2. Mix 7 ml Solution-2 and 5 ml double distilled water in a 12 ml staining jar (must be freshly prepared and must be used within 4 hours), then place slides in the solution mixture. Tightly close the staining jar and wait for 15-30 minutes at room temperature.
3. Rinse slides in Solution-1 two times, 5 seconds each.
4. Rinse slides in distilled water two times, 1 minute each. Distilled water should be renewed frequently.
5. Mix 6.2 ml Solution-3A, 2.5 ml Solution-3B and 2.5 ml Solution-3C in a 12 ml staining jar (must be freshly prepared and must be used within 2 hours), then place slides in the solution mixture. Tightly close the staining jar and wait for 15 minutes at room temperature.
6. Place slides in double distilled water two times, 30 seconds each. with renewed double distilled water.
7. Dip slides in Solution-4 to differentiate.

Note

This differentiation should be carefully performed because elastic staining will fade rapidly. Check under microscope and if over differentiated, return to Step 5.

8. Rinse slides in double distilled water two times, 1 minute each.
9. Rinse slides in Solution-5 for 1 minute.
10. Rinse slides in double distilled water for 5 seconds.
11. Place slides in Solution-6 for 3 minutes. Do not remove excess liquid from the slide.
12. Apply one to four drops of Hito AquaMount™ Mounting Medium on the slide at the end and / or over the tissue (Fig. 1).
13. Carefully lower the coverslip at an angle while gently applying pressure to force any air bubbles away from the tissue and out from under the coverslip (Fig. 2).
14. Gently tilt the slide to remove any medium at the edges of the slide and coverslip. The slide can be viewed immediately.

Warning

Never pour the waste solutions into the sink. Collect the waste solutions in a tightly closed glass or HDPE container and call your safety administration or a licensed professional waste disposal service to dispose of this material.

Note

Slides can be dried at room temperature or at 4°C. Drying at 4°C will increase drying times. Do not heat the slides as this can damage or fade some stains or cause reactions.

Results

Lipid/Fat: Red   Elastic fibers: Black   Nuclei: Gray-blue

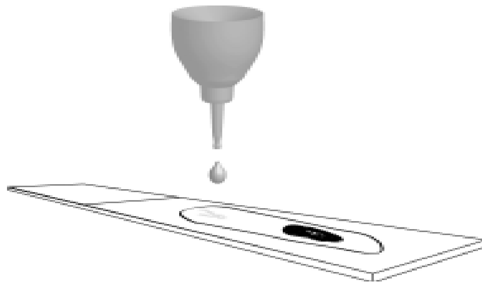

Fig. 1 Apply one to four drops (or more) of Hito AquaMount™ Mounting Medium on the slide at the end and / or over the tissue.

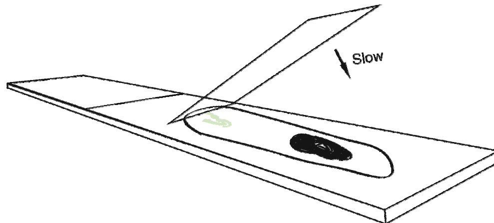

Fig. 2 Draw the coverslip to the edge of the coverslipping medium and then lower it slowly at an angle while applying pressure.

V. References

1 Charpiot, P. et al. Hyperhomocysteinemia induces elastolysis in minipig arteries: structural consequences, arterial site specificity and effect of captopril-hydrochlorothiazide. Matrix Biol 17, 559-574 (1998).

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3 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).

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

5 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: 11/01/2013
Version 2.2

1. Product and Company Information

Product Name	Hito Fat-Elastic Fiber OptimStain™ Kit
Product Number	HTKLE0101
Brand	Hitobiotec
Company Address	Hitobiotec Inc. P.O.Box 7671 Wilmington, DE 19803 USA
Technical Phone:	302-385-6188
Emergency Phone:	302-385-6188

2. Composition and Information on Ingredient

Substance Name	**CAS #**	**SARA 313**
Hito Fat-Elastic Fiber OptimStain™ Kit	None	No

Ingredient Name	**CAS #**	**SARA 313**
WATER	7732-18-5	No
PROPRIETARY COMPONENT(S)	None	No
Oil Red O	1320-06-5	No
2-Propanol	67-63-0	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: 1274 Class: 3 Packing Group: II
Proper shipping name: n-Propanol or Propyl alcohol, normal

IMDG

UN-Number: 1274 Class: 3 Packing Group: II
Proper shipping name: n-Propanol or Propyl alcohol, normal

IATA

UN-Number: 1274 Class: 3 Packing Group: II
Proper shipping name: n-Propanol or Propyl alcohol, normal

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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