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Simple Solution for Your Research

# Hito Von Kossa OptimStain™ Kit

**[Catalog Number: HTKMS1001]**

An easy to use silver staining system
for the morphological characterization
of calcium in frozen or paraffin sections

User Manual
and
Material Safety Data Sheet

FOR IN VITRO RESEARCH USE ONLY

**Hitobiotec Corp.**

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# Index

# I. Introduction

Von Kossa staining has been used to demonstrate deposits of calcium in tissues since the 19th century. The method was originally developed by von Kossa[1], and underwent several modifications[2-4]. The Von Kossa stain is not specific for calcium itself, instead it stains calcium salts like calcium phosphate and calcium carbonate, The silver ions in the staining solution binds with phosphate or carbonate by replacing calcium in a precipitation reaction. The silver salts are then reduced to black metallic silver under strong light illumination. So Von Kossa stain allows indirect visualization of the calcium deposits in tissue samples.

Hito Von Kossa OptimStain™ Kit produces brown to black calcium salts against red cytoplasm and nuclei in just 90 minutes. The developer solution is included in the kit, therefore exposure of the slides to strong light, sunlight, or ultra violet light is not required. This stain kit delivers stable and improved staining quality.

For verification of the staining results, Calcium Positive Control Slides are included in the kit (16-18 days mouse embryo).

Hito Von Kossa OptimStain™ Kit has been tested extensively on the different tissue sections from several species of animals and it is a simple solution for your research.

## II. Kit Contents

**Store Hito Von Kossa OptimStain™ Kit at 4°C in the dark**

| Kit Contents | Standard Kit | Small Kit |
|---|---|---|
| | for 100 slides | for 50 slides |
| Solution-1 | 100 ml | 50 ml |
| Solution-2 | 250 ml | 125 ml |
| Solution-3 | 250 ml | 125 ml |
| Solution-4 | 250 ml | 125 ml |
| Solution-5 | 30 ml | 15 ml |
| Dropping Bottle (for Solution-5) | 1 | 1 |
| Staining Jar (12 ml) | 4 | 4 |
| Calcium Control Slide | 3 | 1 |
| User Manual and MSDS | 1 | 1 |

### Note

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

1. Cryostat or microtome
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. Staining jars for slides wash
4. Resinous mounting medium
5. Light microscope

## III. Tissue Preparation

### For Frozen 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 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 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 15-30 µm thickness on a cryostat with the chamber temperature set at -17°C.
18. Mount the sections on Hito Super-Safe Slides.
19. Air dry slides for 30 minutes at room temperature. Dried sections should be processed as soon as possible but may be stored in a slide box at room temperature for one year.

**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 of 6-15 µm thickness on a cryostat with the chamber temperature set at -17°C.
15. Mount the sections on Hito Super-Safe Slides.
16. Air dry slides for 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 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 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 24-48 hours.
9. After fixation, dehydrate the tissue in a graded ethanol/ water series at room temperature: (for 0.5 $cm^3$ tissue)
    - Flowing tap water for 2 hours
    - 50% ethanol for 2 changes of 2 hours each
    - 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 in the paraffin wax 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 5-11 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 a 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

1. Deparaffin / defat 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 3 minutes during each change.
2. Fill 12 ml Solution-1 in a 12 ml staining jar which is provided in the kit. Place slides in Solution-1 for **one hour** at room temperature. This 12 ml Solution-1 can be reused for 12-16 slides.
3. Rinse the slides in double distilled water 4 times, 1 minute each.
4. Fill 12 ml Solution-2 in a 12 ml staining jar which is provided in the kit. Place slides in Solution-2 for 1-2 minutes at room temperature. This 12 ml Solution-2 can be reused for 4-8 slides.
5. Rinse the slides in double distilled water 2 times, 1 minute each.
6. Fill 12 ml Solution-3 in a 12 ml staining jar which is provided in the kit. Place slides in Solution-3 for 5 minutes at room temperature. This 12 ml Solution-3 can be reused for 4-8 slides.
7. Rinse the slides in double distilled water for 1 minute.
8. Fill 12 ml Solution-4 in a 12 ml staining jar which is provided in the kit. Place slides in Solution-4 for 1 minute at room temperature. This 12 ml Solution-4 can be reused for 4-8 slides.
9. Quickly rinse in 2 changes of double distilled water.
10. Using the dropping bottle (provided in the kit), place a few drops of Solution-5 on the section to fully cover the sections. Allow to stand for 1 minute.
11. Quickly rinse in 2 changes of double distilled water.

12. Rinse slides in 95% ethanol for 2-10 seconds (2-10 dips).
13. Repeat step 12 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 9.

14. Dehydrate in 100% ethanol, 3 changes, 2 minutes each.
15. 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. References


1. von Kossa, J. Über die im Organismus künstlich erzeugbaren Verkalkungen. Ziegler`s Beitr Path Anat 29, 9 (1901).
2. Clark, G. Staining Procedures. (Williams and Wilkins, 1981).
3. Lillie, R. & Fuller, H. Histopathologic technique and practical histochemistry. (McGraw-Hill, 1976).
4. Mallory, F. Pathological techniques: A practical manual for workers in pathological histology including directions for the performance of autopsies and for microphotography. (WB Saunders, 1983).

# VI. Material Safety Data Sheet (MSDS)

Date Updated: 11/02/2016
Version 1.9

## 1. Product and Company Information

| | |
|---|---|
| **Product Name** | Hito Von Kossa OptimStain™ Kit |
| **Product Number** | HTKMS1001 |
| **Brand** | Hitobiotec |
| **Company Address** | Hitobiotec Corp.<br>P.O.Box 7528<br>Kingsport, TN 37664<br>USA |
| **Technical Phone:** | 423-520-6880 |
| **Emergency Phone:** | 423-520-6880 |

## 2. Composition and Information on Ingredient

| **Substance Name** | **CAS #** | **SARA 313** |
|---|---|---|
| Hito Von Kossa OptimStain™ Kit | None | No |

| **Ingredient Name** | **CAS #** | **SARA 313** |
|---|---|---|
| WATER | 7732-18-5 | No |
| Silver Nitrate | 7761-88-8 | Yes |
| Neutral red | 553-24-2 | No |
| Sodium Thiosulfate | 10102-17-7 | No |
| PROPRIETARY COMPONENT(S) | None | No |

## 3. Hazards Identification

**EMERGENCY OVERVIEW**

Harmful by inhalation or in contact with skin or eyes. Possible risk of irreversible damage to skin, mucous membranes, eyes, blood, kidneys and digestive, respiratory, reproductive and central nervous systems.

**HMIS RATING**

HEALTH: 2 FLAMMABILITY: 0 REACTIVITY: 0

**NFPA RATING**

HEALTH: 2 FLAMMABILITY: 0 REACTIVITY: 0

**Potential Health Effects**

| | |
|---|---|
| **I**nhalation | May be harmful if inhaled. Causes respiratory tract irritation. |
| Skin | May be harmful if absorbed through skin. Causes skin irritation. |
| Eyes | Causes eye burns, eye irritation. |
| Ingestion | Toxic if swallowed. Causes burns. |

## 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**

Take off contaminated clothing and shoes immediately. Wash off with soap and plenty of water. Consult a physician.

**In case of eye contact**

Continue rinsing eyes during transport to hospital. 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.

## 6. ACCIDENTAL RELEASE MEASURES

**Personal precautions**

Use personal protective equipment. Avoid dust formation. Avoid breathing dust. Ensure adequate ventilation. Evacuate personnel to safe areas.

**Environmental precautions**

Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.

**Methods for cleaning up**

Pick up and arrange disposal without creating dust. Keep in suitable, closed containers for disposal.

## 7. HANDLING AND STORAGE

**Handling**

Perform experiment in a properly functioning chemical hood, which is vented to the outside. Wear glasses and disposable gloves while handling kit reagents. Wash hands thoroughly after performing the test.

**Storage**

Keep container tightly closed in a dry and well-ventilated place. Store at room temperature, preferably in a cool place.

## 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

**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. Avoid Light.

**Materials to avoid**

Strong oxidizing agents, metals

**Hazardous decomposition products**

Hazardous decomposition products formed under fire conditions. - Hydrogen chloride gas, Mercury/mercury oxides, Potassium oxides, Chromium oxides.

## 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. Causes respiratory tract irritation. |
| **Skin** | May be harmful if absorbed through skin. Causes skin irritation. |
| **Eyes** | Causes eye burns, eye irritation. |
| **Ingestion** | Toxic if swallowed. Causes burns. |

## 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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