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Simple Solution for Your Research

Hito Bielschowsky OptimStain™ Kit

[Catalog Number: HTKNS1126]

An easy to use silver staining system for the
morphological characterization of the
axons, intracellular neurofibrils, and senile plaques

User Manual
and
Material Safety Data Sheet

FOR IN VITRO RESEARCH USE ONLY

Hitobiotec Corp.

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I. Introduction

The Bielschowsky's silver stain is commonly used to demonstrate axons, neurofibrillary tangles, nerve fibers and senile plaques. This staining technique improved on the method developed by Fajersztajn, who initially applied the silver mirror reaction to tissue staining[1]. Max Bielschowsky[2] a German neuropathologist, improved on Fajersztajn's method by using a silver nitrate solution to incubate formalin-fixed frozen sections, followed by an ammoniacal silver solution, a reducing solution and a sodium thiosulfate solution to remove the unreduced silver.

Early modifications of Bielschowsky method were summarized by Beech and Davenport[3]. Further modifications were made by Garven and Gairns[4], Sevier and Munger[5], and Yamamoto–Hirano[6]. However, these Bielschowsky staining procedures use relatively large volume of staining solutions, are costly, time-consuming, and difficult to achieve consistent results.

Hito Bielschowsky OptimStain™ Kit offers a simple solution to these problems. This kit is easy to use with simplified procedures. The users can process slides in large or small quantities without wasting the solutions. The developing step can be controlled slide by slide to achieve optimal staining and differentiation. This kit delivers stable and improved staining quality, with minimal overstains/ background and artifacts when used properly.

Hito Bielschowsky OptimStain™ Kit has been tested on the brains, spinal cords and peripheral nerves from several species of animals and proven to be sensitive for demonstrating the morphological details of axons and intracellular neurofibrils. It is a simple solution for your research.

II. Kit Contents

Store Hito Bielschowsky OptimStain™ Kit

at room temperature

Kit Contents	Standard Kit	Small Kit
	For 100 slides	For 40 slides
Solution-1	50 ml	20 ml
Solution-2	50 ml	20 ml
Solution-3	4 ml	2 ml
Solution-4	250 ml	125 ml
Hito Aqua Barrier PAP Pen	1	1
Staining Jar	2	1
User Manual and MSDS	1	1

Note

Before using Hito Bielschowsky 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, O.C.T. compound, isopentane, ethanol, xylene, double distilled or deionized water
3. Slide (recommend Hito Super-Safe slide Cat# HTHS0101) and coverslips
4. Staining jars for slides wash
5. Resinous mounting medium
6. 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 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 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 (15-30 µm thickness) on a cryostat with the chamber temperature set at -17°C.
18. Mount the sections on the 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 room temperature 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 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)
    - 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 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 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

(Sample protocol for 3 - 4 slides or 6 - 8 mouse brain sections. For more sections, increase the amount used proportionally)

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 3-5 minutes during each change.
2. 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, use a pipet to drop Solution-1 onto each section within the Pap-Pen circle. Fully cover the sections by Solution-1.
3. Place the Solution-1 covered slides in a humidity chamber and incubate for 15-30 minutes at 4°C in the dark (the sensitization step). While waiting for the slides to incubate, carry out Steps 4 - 7.
4. Add **1ml** Solution-1 into 2ml Eppendorf microtube. Add 600µl Solution-2 into the same microtube using a pipet and mix well. Brown precipitate should appear.
5. Add **10 µl** of Solution-2 to the microtube and close the lid, vortex for 3-5 seconds.
6. Repeat Step 5 until the brown precipitate **just** disappears.

Note

Step 6 is very critical. Pay close attention to the brown precipitate. Do not over add Solution-2. The mixture solution should just turn clear by the last addition of 10 µl of Solution-2.

7. After the mixture solution turns clear, add another **10 µl** of Solution-2 and mix well. Pipet 1 ml of the final mixture solution into a new microtube and label it as “Developer”. Save the rest of the mixture solution for Step 9 below.

8. At the end of the incubation from Step 3, rinse the slides in double distilled water for 3 times, 1 minute each. Drain the slides but do not wipe.
9. Using a pipet, drop the saved mixture solution from Step 7 onto each section within the Pap-Pen circle. Fully cover the sections. Place the slides in a humidity chamber and incubate for 15-30 minutes at 4°C in the dark. Dip the slides in double distilled water for one second after completion of incubation.
10. Add 50 ml double distilled water and 200 µl Solution-2 to a 50 ml coplin jar, mix well. Place the slides in the coplin jar until the developing step.
11. Add 60 µl Solution-3 into the 1 ml "Developer" solution set aside from Step 7, mix well. Using a pipet, immediately drop the mixture solution onto the sections within the Pap-Pen circle. Develop for 1 - 8 minutes under the microscope and check for color change. Once a golden brown tissue color is achieved, immediately stop the developing step by placing the slides back into the mixture solution in the coplin jar from Step 10 for 1 minute.
12. Place slides in Solution-4 in a 12 ml staining jar (provided in the kit) for 3 minutes at room temperature.
13. Rinse the slides in double distilled water twice, 2 minutes each.
14. Dehydrate the slides in 50% ethanol, 75% ethanol, 95% ethanol and 100% ethanol, with 2 changes in each step, 3-5 minutes during each change.
15. Clear in xylene, 2 times, 4 minutes each. Apply coverslip over sections using xylene based resinous mounting medium.
16. Allow to dry. The slide can be viewed after drying by bright field microscopy.

V. References

1. Fajersztajn, J. Ein neues Silberimprägnationsverfahren als Mittel zur Färbung der Axencylinder. Neurologisches Zentralblatt 20, 98-107 (1901).
2. Bielschowsky, M. Eine Modifikation meines Silverimprägnationsverfahrens zur Darstellung der Neurofibrillen. J für Psychologie Neurologie 12, 135–137 (1908).
3. Beech, R. H. a. D., H.A. The Bielschowsky Staining Technic a Study of the Factors Influencing Its Specificity for Nerve FIBERS. Biotechnic & Histochemistry 8, 11-29, (1933).
4. Garven, H. S. & Gairns, F. W. The silver diammine ion staining of peripheral nerve elements and the interpretation of the results: with a modification of the Bielschowsky-Gros method for frozen sections. Q J Exp Physiol Cogn Med Sci 37, 131-142 (1952).
5. Sevier, A. C. & Munger, B. L. Technical Note: A Silver Method for Paraffin Sections of Neural Tissue. J Neuropathol Exp Neurol 24, 130-135 (1965).
6. Yamamoto, T. & Hirano, A. A comparative study of modified Bielschowsky, Bodian and thioflavin S stains on Alzheimer's neurofibrillary tangles. Neuropathol Appl Neurobiol 12, 3-9 (1986).

VI. Material Safety Data Sheet (MSDS)

Date Updated: 12/01/2015
Version 1.9

1. Product and Company Information

Product Name	Hito Bielschowsky OptimStain™ Kit
Product Number	HTKNS1126
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 Bielschowsky OptimStain™ Kit	None	No

Ingredient Name	**CAS #**	**SARA 313**
WATER	7732-18-5	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

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.

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. Read Terms & Conditions page on our website for additional terms and conditions of sale.

Hitobiotec Corp.

P.O.Box 7528
Kingsport, Tennessee
U.S.A.

Phone: 423-520-6880

Email: info@hitobiotec.com

www.hitobiotec.com