

---

---

**BODIAN'S METHOD - NERVE FIBERS**

---

---

**PURPOSE:** For demonstrating nerve fibers.

**PRINCIPLE:** Protargol-S (silver proteinate) is used with the addition of copper metal. The copper replaces the silver in the connective tissue, allowing a greater differentiation between the nerve fibers and the connective tissue. The silver is reduced with hydroquinone to the visible metallic form. The sections are toned with gold chloride, the gold chloride is reduced with oxalic acid, increasing the deposit of metallic gold on the sections.

**CONTROL:** Normal cerebral cortex.

**FIXATION:** 10% formalin

**TECHNIQUE:** Cut paraffin section at 6m. Allow slide to dry overnight at 37°C.

**EQUIPMENT:** Acid clean glassware. 37°C oven.

**REAGENTS:**

**Reducing Solution:**

Hydroquinone	0.5 gm
Sodium sulfite	2.5 gm
Distilled water	50.0 ml

Make fresh, discard after use.

**CAUTION:** Avoid contact and inhalation.

**Copper sheet:**

Copper sheet	5.0 gm (1/2")
Hydrochloric acid	

Wash the copper sheet in the acid, rinse well in tap water and 3 changes of distilled water.

**CAUTION:** Corrosive.

**Protargol-S Solution:**

Protartol-S	0.5 gm
(Purchased through Polysciences Inc.)	

Distilled water	50.0 ml
-----------------	---------

Copper sheet, acid cleaned

Use acid cleaned glassware. Add the copper sheet and the distilled water in a coplin jar. Gradually sprinkle the Protargol-S on top of the water. Do not mix, allow the solution to sit undisturbed until dissolved. Make fresh, discard after use.

**0.5% Oxalic Acid:**

Oxalic acid 1.0 gm  
Distilled water 200.0 ml

Mix well. Stable for 1 year.

**5% Hypo:**

Sodium thiosulfite 5.0 gm  
Distilled water 100.0 ml

Solution is stable for 1 year.

**CAUTION:** Avoid contact and inhalation

**SAFETY:** Wear gloves, goggles and lab coat. Avoid contact and inhalation.

Hydrochloric acid: strong irritant to skin, eyes and respiratory system. Target organ effects via inhalation on skin, respiratory, reproductive and fetal systems.

Oxalic acid; Toxic on inhalation and ingestion. Can cause death on ingestion of 5gm. Target organ effects on cardiovascular system and kidneys on ingestion. Corrosive. Irritant to skin, eyes and mucous membranes.

Sodium thiosulfate: Toxic on ingestion. Can irritate the stomach. Irritant to skin, eyes and respiratory tract.

Hydroquinone; irritant to skin and eyes. Toxic by inhalation and ingestion. Chronic exposure has caused bladder cancer in mice.

**PROCEDURE:**

1. Deparaffinize and hydrate to distilled water.
2. Place in Protargol solution, 37°C oven, overnight.
3. Rinse in distilled water, two quick dips.
4. Reducing solution, 5 minutes.
5. Wash in tap water, rinse in distilled.
6. 0.5% Gold chloride, 3-5 minutes, or until gray.
7. Distilled water.
8. 0.5% Oxalic acid, 10 minutes, observe under the microscope for black fibers.
9. Distilled water.
10. 5% hypo, 3 minutes.
11. Wash in tap water.
12. Dehydrate, clear in xylene, coverslip.

**RESULTS:**

Nerve fibers        black

**REFERENCE:**

Lillie R.D., Histopathologic Technic and Practical Histochemistry, 4th Ed, 1976, pp771-772, McGraw-Hill, NY

Carson F, Histotechnology: A Self-Instructional Text, 1990, pp172-174, ASCP, Ill

Crookham, J, Dapson, R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech

Prepared: \_\_\_\_\_ By: \_\_\_\_\_

Approved: \_\_\_\_\_ By: \_\_\_\_\_

# PROCEDURE CARD

## BODIAN'S METHOD - NERVE FIBERS

---

**CONTROL:** Normal cerebral cortex.

**TECHNIQUE:** Cut paraffin section at 6m. Allow slide to dry overnight in a 60°C oven.

### PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. Place in Protargol solution, 37°C oven, overnight.
3. Rinse in distilled water, two quick dips.
4. Reducing solution, 5 minutes.
5. Wash in tap water, rinse in distilled.
6. 0.5% Gold chloride, 3-5 minutes, or until gray.
7. Distilled water.
8. 0.5% Oxalic acid, 10 minutes, observe under the microscope for black fibers.
9. Distilled water.
10. 5% hypo, 3 minutes.
11. Wash in tap water.
12. Dehydrate, clear in xylene, coverslip.

### RESULTS:

Nerve fibers black

#### Copper sheet:

Copper sheet 5.0 gm (1/2")  
Hydrochloric acid

Wash the copper sheet in the acid, rinse well in tap water and 3 changes of distilled water.

**CAUTION: Corrosive.**

#### Protargol - S Solution:

Protargol-S 0.5 gm  
(purchased through Polysciences Inc.)  
Distilled water 50.0 ml  
Copper metal, acid cleaned

Use acid cleaned glassware. Add the copper metal and the distilled water in a coplin jar. Gradually sprinkle the Protargol-S on top of the water. Do not mix, allow the solution to sit undisturbed until dissolved. Make fresh, discard after use.

#### 5% Hypo:

Sodium thiosulfite 5.0 gm  
Distilled water 100.0 ml

Solution is stable for 1 year.

#### Reducing Solution:

Hydroquinone 0.5 gm  
Sodium sulfite 2.5 gm  
Distilled water 50.0 ml

Make fresh, discard after use.

**CAUTION: Avoid contact and inhalation.**

#### 0.5% Gold Chloride:

Gold chloride 1.0 gm  
Distilled water 200.0 ml

Mix well, store in acid clean bottle, in the refrigerator. Stable.

**CAUTION: Avoid contact and inhalation.**

#### 0.5% Oxalic Acid:

Oxalic acid 1.0 gm  
Distilled water 200.0 ml

Mix well. Stable for 1 year.

**CAUTION: Avoid contact and inhalation.**

**0.5% Oxalic Acid:**

Oxalic acid 1.0 gm  
Distilled water 200.0 ml

Mix well. Stable for 1 year.

**CAUTION: Avoid contact and inhalation.**

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

EXPIRATION: \_\_\_\_\_

**0.5% OXALIC ACID**

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

**0.5% Gold Chloride:**

Gold chloride 1.0 gm

Distilled water 200.0 ml

Mix well, store in acid clean bottle,  
in the refrigerator. Stable.

**CAUTION: Avoid contact and inhalation.**

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

EXPIRATION: \_\_\_\_\_

**0.5% GOLD CHLORIDE**

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_