

## **Protargol-S™**

### *Brand of Silver Albumose (Silver Protein) Certified by the Biological Stain Commission*

#### **Intended Use:**

Silver albumose here supplied is intended for the qualitative procedure of staining nerve tissue by a modified Bodian technique.

#### **Method History:**

The first procedure utilizing Protargol for staining nerve fibers was developed by Bodian (1936).<sup>1</sup> This stain consisted of 1% Protargol plus 5g (per 50 ml) of clean, bright granulated copper. In 1939, Davenport, et al., reported on a two-hour method which included 5% aqueous AgNO<sub>3</sub> for one hour followed by 0.2% Protargol for one hour.<sup>2</sup> A procedure which showed axons and sheaths on the same section was developed by Foley (1943).<sup>3</sup> Frozen or celloidin sections were stained with Protargol and counterstained for myelin sheaths with fast green. In 1947, Davenport, et al., proposed the use of a mixture of 0.5% Protargol and 0.1% fast green FCF as a silver-dye staining medium.<sup>4</sup>

Of the many methods used to demonstrate nerve endings and nerve fiber tissue, most are a modification of the original Bodian Stain. The technique presented here is a modified Bodian Stain (Russell, 1973).<sup>5</sup>

#### **Principle:**

When nerve tissue is soaked in silver nitrate, the nature of the reaction is such that the nerve cells that become impregnated with silver stand out against a clear background. The use of copper with silver staining prevents the tissue from becoming permeated with silver, which tends to obliterate cellular detail. Acidic dyes are used as counterstains because their affinity for smooth muscle and connective tissue blocks them against staining with Protargol and therefore gives more selective nerve fiber stains.

#### **Reagents:**

Protargol-S™ is a light tan to dark brown odorless powder. The silver albumose solution is prepared by sprinkling 1g Protargol-S™ on the surface of 100ml of distilled water. Allow the granules to dissolve without stirring and without heat. Pour the Protargol-S™ solution into a Coplin jar containing 6g of bright metallic copper (granules, wire or foil). If staining is carried out in Coplin jars, this will be 3g of copper per jar, since a Coplin jar usually holds about 50ml solution. The copper may be reclaimed and used again.

Hydroquinone solution (which must be made up fresh) is prepared by mixing 1g hydroquinone with 5ml 40% formaldehyde (commercial Formalin) with 100ml distilled water. Van Gieson's solution is prepared by mixing 2.5ml of 1% aqueous acid fuchsin with 97.5ml of saturated picric acid. Solutions should be made up daily. Used portions of Protargol-S™ should be discarded. When not in use keep container of Protargol-S™ tightly capped. **Caution:** Protargol-S™ is intended for *in vitro* diagnostic use.

#### **Materials and Solutions:**

**Materials provided** (prepared as above): Protargol-S™ Powder

#### **Additional materials and solutions required:**

1. Copper slot, wire or foil
2. Gold chloride solution 0.2% (Hypo) 5%
3. Oxalic acid solution
4. Sodium thiosulfate sol
5. Xylene
6. Alcohol
7. Paraffin
8. Distilled water

#### **Instruments used:**

1. Automatic tissue processor for preparing nerve tissue.
2. Microtome for sectioning tissue.

#### **Specimen Collection:**

In the case of human nerve tissue, samples should be obtained by a physician who is qualified by virtue of training and experience to perform this surgical procedure. The specimen should be placed in a fixative such as 10% buffered Formalin or Bouin's solution immediately after removal.

#### **Fixation:**

The fixation for the Bodian Stain is of initial concern. The use of 10% Formalin is satisfactory if the pH is 6.8 or 7.0. Bouin's solution can be used as a substitute fixative.

**Procedure for Staining:**

1. Section paraffin block at 5-6 microns. Thin sections allow for best results.
2. Dry sections in 60°C oven for 2 hours.
3. Deparaffinize in three changes of xylene.
4. 100% alcohol, one change.
5. 95% alcohol, one change.
6. Place deparaffinized sections in Protargol-S™ solution, 37°C, 12 to 30 hours.
7. Rinse in six changes of distilled water.
8. Reduce in hydroquinone/Formalin solution, 15 minutes.
9. Rinse in six changes of distilled water.
10. Tone in 0.2% gold chloride solution, 10-15 minutes until light gray color shows.
11. Rinse in six changes of distilled water.
12. Develop silver in 2% oxalic acid solution, 2 to 3 minutes. Slides should be checked with microscope after 1 minute. Continue development until nerve fibers are sharp.
13. Rinse in six changes of distilled water.
14. Fix silver in Hypo, 5 to 10 minutes.
15. Rinse in six changes of distilled water.
16. Counterstain with Van Gieson's solution, 1 to 3 minutes. Sharp contrast will be increased if this is done.
17. Dehydrate in 95% alcohol, one change; 100% alcohol, two changes.
18. Clear in xylene, two changes.
19. Mount.

**Typical Results:**

Through the microscope nerve fibers and endings will appear black and the background will appear yellow green.

**Caution:**

1. Make fresh Protargol-S™ solution just before use. Solutions that stand 2 to 3 days sometimes lose their alkalinity.
2. The pH of the Protargol-S™ staining solution should test about 8.5 when diluted 1:20 with distilled water. This solution gives a just perceptible reddening with phenolphthalein indicator.
3. The action of the oxalic acid bath, after gold toning, should be watched carefully and sections should not be allowed to remain in it until they have become too dark.

**Ordering Information:**

Cat. #	Description	Size
01070	Protargol-S™	5g 25g

To Order:

In The U.S. Call: 1-800-523-2575 • 215-343-6484

In The U.S. FAX: 1-800-343-3291 • 215-343-0214

In Germany Call: (49) 6221-765767

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Order online anytime at [www.polysciences.com](http://www.polysciences.com)

**References:**

1. Bodian, D., Anat. Rec., **65**, 89-97 (1936).
2. Davenport, H.A., et al., Stain Tech., **14**, 22-26 (1939).
3. Foley, J.O., Stain Tech., **18**, 27-33 (1943).
4. Davenport, H.A., et al., Stain Tech., **22**, 41-49 (1947).
5. Russell, R., Lab. Med., **4**, 40 (1973).

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