

BODIAN'S METHOD FOR NERVE FIBERS AND NERVE ENDINGS

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of nerve fibers.
PRINCIPLE:	Silver proteinate is taken up by nerve fibers while the copper metal in the solution inhibits the connective tissue from binding the silver. The silver is then reduced by the Hydroquinone Solution to the visible form. Gold Chloride displaces nonreacted silver ions and then is reduced by Oxalic Acid to increase staining intensity. Sodium Thiosulfate removes any loose metallic ions and Aniline Blue is used as a counterstain.
CONTROL:	Normal cerebral cortex, nerve <i>Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs031.</i>
SPECIMEN PREPARATION:	Formalin fixed, paraffin embedded sections cut at 6 micrometers
SOLUTIONS:	<ol style="list-style-type: none"> 1. Protargol Dry Pack Item# s2014-3x1GM See directions below.* 2. Bodian's Reducing Solution, See directions below.* 3. Gold Chloride 1% Aqueous Item# s203 4. Oxalic Acid 2% Aqueous Item# s2012 5. Sodium Thiosulfate 5% Aqueous Item# s1895 6. Aniline Blue Bodian's Method Item# s117 7. Copper Wire Item# P206-5GM <p><i>Solutions can be purchased separately from Poly Scientific.</i></p>
NOTES:	<p>*These solutions must be made fresh: Protargol Solution: Protargol Powder 3x1 g, Distilled Water 3x100 mL. Sprinkle 1 gram Protargol onto surface of 100 mL of distilled water. Allow it to dissolve. Discard after use. Bodian's Reducing Solution: Hydroquinone 3x1 g, Formaldehyde 3x5 mL, Distilled Water 3x100 mL. Mix 1 gram of Hydroquinone & 5 mL of Formaldehyde with 100 mL of distilled water. Expiration date: 2-3 weeks after reconstitution.</p>
REFERENCE:	Luna, L.G. "Luna's Modification of Bodian's Method for Axons and Nerve Endings". <i>Amer. J. Medical Technology</i> . 30:355, 1964.

STAINING PROCEDURE:

1. Deparaffinize and hydrate to water.
2. Place slides in Protargol Solution and add 4-6 grams of clean copper wire per 100 mL of solution. Let stand at 37°C for 48 hours.
Note: Copper wire must be cleaned with a solution comprised of 15 mL Hydrochloric Acid and 5 mL of Nitric Acid, then rinsed in distilled water several times. The copper wire may be cleaned and reused.
3. Rinse distilled water, 3 changes.
4. Place in Bodian's Reducing Solution for 10 minutes.
5. Rinse in distilled water, 3 changes.
6. Tone in Gold Chloride 1% Aqueous for 5 minutes.
7. Rinse in distilled water, 3 changes.
8. Develop in Oxalic Acid 2% Aqueous under the microscope until background is gray and nerve fibers appear clearly, approximately 3-5 minutes.
9. Rinse in distilled water, 3 changes.
10. Place in Sodium Thiosulfate 5% Aqueous for 5 minutes.
11. Rinse in distilled water.
12. Counterstain, if desired, with Aniline Blue Solution, 2 or 3 quick dips, to give a light blue background.
13. Dehydrate in 95% Alcohol, Absolute Alcohol and clear in Xylene, 2 changes each.
14. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Nerve Fibers	Black
Background	Blue
Nuclei	Black

Poly Scientific R&D Corp.

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