REAGENT ASSEMBLY KIT
ITEM# mw019

BIELSCHOWSKY'S METHOD FOR NEUROFIBRILS (Microwave)

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of senile plaques and fibrillary tangles.
PRINCIPLE:	Nerve fibers are first sensitized with a Silver Solution and then they absorb silver from the Diamine Silver Solution. The silver is reduced to metallic silver by Formalin and the excess is removed by Thiosulfate.
CONTROL:	Cerebral cortex of a known case of Alzheimer's *Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs031.*
SPECIMEN PREPARATION:	15% Buffered Formalin, for at least 2 weeks, fixed, paraffin embedded sections cut at 8 micrometers
SOLUTIONS:	1. Silver Nitrate 1% Aqueous Item# s1888 2. Ammonium Hydroxide Concentrated Item# c804 3. Silver Nitrate 5% Aqueous Item# s1890 4. Ammonium Hydroxide 1% Solution Item# s2338 5. Sodium Thiosulfate 2% Aqueous Item# s280 6. Bielschowsky's Developing Solution Item# s2788 *Solutions can be purchased separately from Poly Scientific.*
NOTES:	Use acid cleaned glassware. Do not crowd slides in the staining container. Use the same silver solution throughout the technique. *Variations in timing may occur due to the power wattage of the microwave oven. Provided times and power levels are based on 1000 watt microwave oven.*
REFERENCE:	Luna, Lee G. Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts. American Histolabs Inc. Gaithersburg, MD. 1992. p. 484.

STAINING PROCEDURE:

1. Deparaffinize and hydrate slides to distilled water.
2. Place slides in 40 mL of Silver Nitrate 1% Aqueous in a plastic coplin jar. Microwave at power level 3 (180 W) for 1 minute. Dip slides several times in warm solution and let set for 15 minutes.
3. Rinse slides in distilled water.
4. To make Ammoniacal Silver Nitrate, pour the warm Silver Nitrate 1% from step 2 into a 125 mL flask. Add Ammonium Hydroxide Concentrated drop by drop, constantly shaking, until the solution clears. Next add Silver Nitrate 5%, drop by drop, shaking constantly until the solution becomes slightly cloudy.
5. Pour Ammoniacal Silver Nitrate Solution prepared in step 4 into a plastic coplin jar. Add slides and microwave at power level 3 (180 W) for 1 minute. Dip slides up and down in the warm solution and let set for 15 minutes.
6. Place slides directly into Ammonium Hydroxide 1% Solution for not more than 20 seconds.
7. Add 3 drops of the Developer into the Ammoniacal Silver Nitrate Solution from step 5 and mix with glass rod. Place slides immediately into the solution for 2-3 minutes or until the sections turn brown. *Note:* The solution will turn a gray color and a sheen of silver will form on the sides of the coplin jar and sometimes on the glass slides but not on the tissue sections.
8. Place slides in Ammonium Hydroxide 1% Solution for no longer than 15 seconds.
9. Rinse slides in 3 changes of distilled water.
10. Carefully wipe off silver from both sides of slides. Do not touch tissue section.
11. Place slides in Sodium Thiosulfate 2% Aqueous for 30 seconds.
12. Rinse slides in 4 changes of distilled water.
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:

Neurofibrillary Tangles and Neuritic Plaques of Alzheimer's Disease	Dark Brown to Black
Neuromelanin, Lipofuscin	Densely Black & Coarsely Granular
Axons	Brown to Black
Amyloid	Dense Black Cores
Background	Yellow-Brown