

MODIFIED BIELSCHOWSKY'S STAIN KIT PROCEDURE

Item# **KTBIE**

(Revised 04/19/11)

KIT COMPONENTS INCLUDED:	Pint 20% SILVER NITRATE	100ml. 5% SODIUM THIOSULFATE
	6ml. STOCK DEVELOPER SOL "A"	6ml. STOCK DEVELOPER SOL "B"
	6ml. STOCK DEVELOPER SOL "C"	

PRINCIPLE: This stain kit demonstrates axons, intracellular neurofibrils and senile plaques.

SPECIMEN: Frozen sections cut at 5 to 7 microns. Formalin fixed paraffin sections cut at 8 microns, deparaffinized using Xylene or a Xylene Substitute and hydrated through graded alcohols and absolute alcohol to Distilled water.

QUALITY CONTROL: American MasterTech Recommended Control Slide: Alzheimer, CSA0225P

AMMONIACAL SILVER SOLUTION:

Place 20 ml of **20% SILVER NITRATE** in chemically cleaned glassware or a virgin plastic container and gradually add concentrated Ammonium Hydroxide (*not included in kit*) while carefully agitating container until no precipitate remains.

DEVELOPER SOLUTION:

Prepare just before use by placing 50 ml of Distilled Water in chemically cleaned glassware or a virgin plastic container, then add in the following order: 8 drops of **Stock Developer Sol. "A"**, 8 drops of **Stock Developer Sol. "B"** and 4 drops of **Stock Developer Sol. "C"**. **NOTE:** Mix DEVELOPER SOLUTION thoroughly following each addition.

AMMONIA WATER:

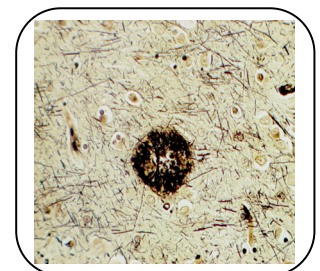
Prepare **AMMONIA WATER** by adding a few drops of concentrated Ammonium Hydroxide (*not included in kit*) to 25 ml of Distilled Water (approximately 0.25% Ammonium Hydroxide in Water).

PROCEDURE:

1. Rinse slide thoroughly through 2 changes of Distilled water.
2. In a waterbath place slide in preheated **20% SILVER NITRATE** for 15 minutes at 40° C. Do not reuse this solution!
3. Rinse slide thoroughly through 3 changes of Distilled water.
4. Place slide in preheated **AMMONIACAL SILVER SOLUTION** for 10 minutes at 40° C. Do not reuse this solution!
5. Drain slide and without rinsing place in room temperature **DEVELOPER SOLUTION**. Agitate slide for 3 seconds to 30 seconds; remove slide when a golden brown tissue color is achieved. Do not reuse this solution!
6. Quickly place slide in **AMMONIA WATER** for 30 seconds to stop the developing process.
7. Rinse slide thoroughly through 3 changes of Distilled water.
8. Place slide in **5% SODIUM THIOSULFATE** for 2 minutes.
9. Rinse slide thoroughly in Distilled water.
10. Dehydrate slide through 3 changes of fresh Absolute Alcohol.
11. Clear slide through 3 changes of fresh Xylene or Xylene Substitute.
12. Coverslip slide using a permanent mounting media.

RESULTS:

Intracellular Neurofibrils (neurofibrillary tangles),
axons and senile plaques: **GRAY TO BLACK**
Nuclei and background: **YELLOW TO GOLD**



Human Brain

REFERENCE: Mirra SS Hart MN Terry RD: Making the Diagnosis of Alzheimer's Disease, Arch Pathol Lab Med. 1993;117: 132 - 144. Luna LG: Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts; American Histolabs, Inc. 1992: 483 - 485.

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