



# Instructions For Use

## BSK-IFU

205 South 600 West Logan, Utah 84323, U.S.A. – Tel. (800) 729-8350 – Tel. (435) 755-9848 – Fax (435) 755-0015 – www.scytek.com Rev. 4, 7/19/2022

## Bielschowsky's Stain Kit (Modified)

### Description and Principle

The Bielschowsky's Stain Kit (Modified) is designed for histological visualization of nerve fibers, neurofibrillary tangles and senile plaques in Alzheimer's disease.

Tissue section is impregnated with an ammoniacal silver solution. Silver that is deposited on nerve fibers, neurofibrillary tangles and senile plaques is reduced to visible metallic silver by formalin. Sodium thiosulfate removes unreduced silver from the tissue.

### Expected Results

Axons:	Black
Neurofibrillary Tangles:	Black
Senile Plaques:	Black
Nuclei:	Dark Brown
Background:	Yellow to Light Brown

### Kit Contents

Kit Contents	Storage
1. Silver Nitrate Solution (20%)	2-8°C
2. Formalin Solution (20%)	18-25°C
3. Citric Acid Solution (Bielschowsky's)	18-25°C
4. Nitric Acid Solution (Bielschowsky's)	18-25°C
5. Sodium Thiosulfate Solution (5%)	18-25°C

### Required but Not Included:

Concentrated Ammonium hydroxide (25-30%)

### Suggested Controls *(not provided)*

Cerebral cortex (cut 8-10µm).

### Uses/Limitations

For In-Vitro Diagnostic use only.

Do not use if reagents become cloudy or precipitate

Do not use past expiration date.

Use caution when handling reagents.

Non-Sterile

Intended for FFPE sections cut at 5-10µm.

This procedure has not been optimized for frozen sections.

Frozen sections may require protocol modification.

### Storage

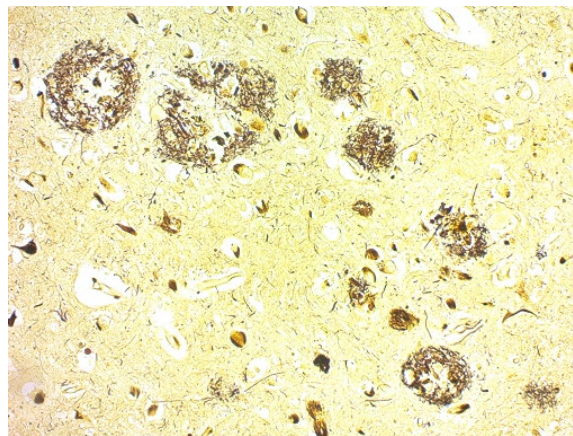
Mixed storage conditions. Store according to individual label instructions.

### Safety and Precautions

Please see current Safety Data Sheets (SDS) for this product and components GHS classification, pictograms, and full hazard/precautionary statements.

### Important Notes

1. All glassware used in this procedure should be chemically cleaned and rinsed thoroughly in distilled water.
2. Do not use metal forceps to remove slides from reagents. Use plastic forceps only.
3. Equilibrate all reagents to room temperature prior to use.



Human Brain with Alzheimer's stained with Bielschowsky's Stain Kit (modified)

### Preparation of Reagents Prior to Beginning:

1. Prepare working **Ammoniacal Silver Solution (used in step 4)** using chemically cleaned glassware in a chemical fume hood as follows: Pour 25-50ml of Silver Nitrate Solution (20%) into container (volume used is dependent on amount required to adequately fill staining container). Add concentrated ammonium hydroxide (25-30%) (not included in kit); drop by drop, while swirling the flask continuously, until precipitate just dissolves and the reagent goes clear. NOTE: If a small excess of ammonium hydroxide (25-30%) is added and solution will not go completely clear, filter the solution using a paper filter prior to use!

**Note: Use extreme care in preparation and use of Ammoniacal Silver Solution. Use mixture once and dispose. Dispose of waste observing all local, state and federal laws.**

2. Prepare **Developer Solution (used in step 8)** using chemically cleaned glassware immediately prior to use as follows:

Swirl carefully throughout mixing steps.

50 ml	Distilled Water
8 Drops	Formalin Solution (20%)
8 Drops	Citric Acid Solution (Bielschowsky's)
4 Drops	Nitric Acid Solution (Bielschowsky's)

3. Prepare working **Ammonia Water (used in step 8)** by mixing 320µl (8 drops) of concentrated Ammonium hydroxide (25-30%) (not included) in 50 ml of distilled water.

### Procedure

#### Preheat waterbath to 40°C

1. Deparaffinize sections if necessary and hydrate to distilled water.
2. Place a chemically cleaned staining jar containing 25ml of Silver Nitrate Solution (20%) in waterbath and allow temperature to equilibrate for 10 minutes.
3. Place slide in warmed Silver Nitrate Solution (20%) and incubate for 15 minutes at 40°C.

4. During incubation place Ammoniacal Silver Solution in waterbath in allow temperature to equilibrate.
5. Remove slide from Silver Nitrate Solution (20%) and rinse in 4 changes of distilled water.
6. Place slide in warmed Ammoniacal Silver Solution and incubate for 10 minutes at 40°C.
7. Remove slide from Ammoniacal Silver Solution, shake off excess and place directly into Developer Solution. Agitate gently until tissue section takes on a yellow/brown hue (5-20 seconds).
8. Remove slide from Developer Solution and immediately place in Ammonia Water for 30 seconds.
9. Rinse in 4 changes of distilled water.
10. Apply adequate Sodium Thiosulfate Solution (5%) to completely cover tissue section and incubate for 2 minutes.
11. Rinse in 4 changes of distilled water.
12. Dehydrate in 3 changes of absolute alcohol for 2 minutes each.
13. Clear, and mount in synthetic resin.

### **References**

1. Carson, F.L., 1996, Histotechnology; A Self-Instructional Text, 2nd Edition. ASCP Press, Chicago, IL. Pages 200-205.
2. Mirra, S. S., M. Noel Hart, and R. D. Terry. "Making the diagnosis of Alzheimer's disease. A primer for practicing pathologists." *Archives of pathology & laboratory medicine* 117, no. 2 (1993): 132-144.



ScyTek Laboratories, Inc.  
205 South 600 West  
Logan, UT 84321  
435-755-9848  
U.S.A.



Emergo Europe  
Prinsessegracht 20  
2514 AP The Hague, The Netherlands