

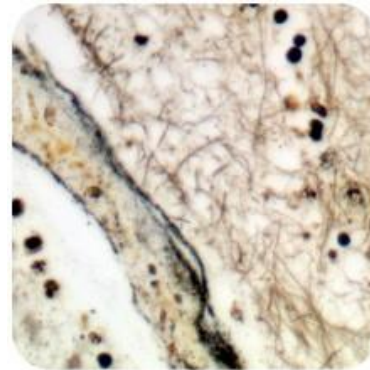


Bielschowsky's Stain Kit (Modified)

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

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

Uses/Limitations: Not to be taken internally.
For In-Vitro Diagnostic use only.
Histological applications.
Do not use if reagents become cloudy.
Do not use past expiration date.
Use caution when handling reagents.
Non-Sterile.



Control Tissue: Cerebral cortex (cut 8-10µm)


Availability:

<u>Kit Contents</u>	<u>Volume</u>	<u>Storage</u>
Silver Nitrate Solution (20%)	500 ml	2-8°C
Formalin Solution (20%)	8 ml Dropper	18-25°C
Citric Acid Solution (Bielschowsky's)	8 ml Dropper	18-25°C
Nitric Acid Solution (Bielschowsky's)	8 ml Dropper	18-25°C
Sodium Thiosulfate Solution (5%)	125 ml	18-25°C

Required but Not Included: Concentrated Ammonium Hydroxide.

Storage: Mixed storage conditions. Store according to individual label instructions.

Precautions: Avoid contact with skin and eyes.
Harmful if swallowed.
Follow all Federal, State, and local regulations regarding disposal.

Storage: 2° C  25° C

Mixed Storage Conditions.
Separate Contents.



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 (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 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 (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.

Storage: 2° C  25° C

Mixed Storage Conditions.
Separate Contents.