VitroView™ Bielschowsky's Silver Stain Kit

SKU No. VB-3015

Introduction

Bielschowsky's Silver Stain Kit is designed for the staining of nerve fibers and the detection of neurites and neurofibrillary tangles. Nerve fibers are sensitized with a silver solution. The sections are treated with ammonical silver, then reduced to visible metallic silver. Axons, neurofibrillary tangles and senile plaques will be stained black, and the background will be yellow to brown.

Kit Components

VB-3015-1 Silver Nitrate Solution ----------100 ml
VB-3015-2 Developer Stock Solution A ----------2 ml
VB-3015-3 Developer Stock Solution B ----------1 ml
VB-3015-4 Concentrated ammonium hydroxide ----------15 ml
VB-3015-5 5% Sodium Thiosolfate ----------100 ml

Storage:

Store at 2-8ºC.

Protocol

1. Let the kit stand at room temperature for 30 min before use.
2. Section Preparation:
    a. Paraffin-embedded tissue sections: Deparaffinize and hydrate with 70% ethanol. Rinse in distilled water.
    b. Frozen sections: Dry sections for 5-10 minutes. Rinse in dH2O.
3. Stain slides by incubating in pre-warmed (40 ºC) silver nitrate solution for 15 minutes. Sections should turn light brown (thin sections) or dark brown (thick sections) in color.
4. Wash 3 × in dH2O.
5. Prepare ammonium silver solution.
    a. Add concentrated ammonium hydroxide one drop at a time to the silver nitrate solution until the precipitate that forms clears. Excess ammonium hydroxide may cause a precipitate and result in a poor impregnation of the fibers.
    b. If ammonium hydroxide is added too quickly, add a few more drops of silver nitrate solution to help clear the precipitate.
6. Incubate slides in the ammonium silver solution in a 40 ºC oven for 30 minutes or until sections become dark brown.
7. Prepare developer working solution by mixing the following components in 50ml dH2O. This solution should be prepared fresh for each staining experiment and discarded after use.
    a. 400µl Developer Stock Solution A
    b. 80µl Developer Stock Solution B
    c. 400µl concentrated ammonium hydroxide
8. Place slides directly (do not wash slides) in developer working solution for 1 minute or less. The staining reaction can occur very quickly, so we recommend first determining the appropriate incubation time using a test slide and visualizing it under a microscope.
9. Dip slides for 1 minute in 1% ammonium hydroxide solution (1ml concentrated ammonium hydroxide in 100ml dH2O) to stop the silver reactio.
10. Wash slides 3 × with dH2O.
11. Place slides in 5% sodium thiosulfate solution for 5 minutes.
12. Wash slides 3 × with dH2O.

13. Dehydrate with 95% ethanol.
14. Wash slides 2 × 3 minutes in 100% ethanol.
15. Incubate slides 3× 5 minutes in xylene.
16. Mount coverslip onto glass slide with Permount or other suitable organic mounting medium.

Expected results

- Axons, neurofibrillary tangles and senile plaques ------- black
- Background ----------yellow to brown

Positive Controls

Brain tissue

Note

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Precautions

Handle with care. Avoid contact with eyes, skin and clothing. Do not ingest. Wear gloves.