

# Bielschowsky (for neurofibrils), Kit

Reference: AP0359



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## INTENDED USE AND PRESENTATION:

For *in vitro* diagnostic use.

AP0359. 100 Tests.

## APPLICATIONS:

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

The interpretation of the stain results is the full responsibility of the user. Any experimental result must be confirmed by a medically established diagnostic product or procedure.

## PRODUCT COMPOSITION:

The Bielschowsky (for neurofibrils) kit is composed by:

Reagent	Name	Volume	Storage
A	Silver nitrate solution	4 x 45 mL	2-8 °C
B	Formalin solution	4 mL	15-25 °C
C	Citric acid solution	4 mL	15-25 °C
D	Nitric acid solution	4 mL	15-25 °C
E	Sodium thiosulfate solution	2 x 30 mL	15-25 °C
F	Concentrated ammonium hydroxide	2 x 45 mL	15-25 °C

## METHODS AND PROCEDURE:

**Principles of the method:** Impregnations methods are extensively used in neurohistology and there isn't at present any way of selectively coloring axons and dendrites with dyes in routine preparations. Silver impregnations methods are based on the following principle: the silver which is present in the ammoniacal solution in form of complex hydrosoluble oxide -  $[\text{Ag}(\text{NH}_3)_2]_2\text{O}$  is reduced by some cellular components to black, stable and insoluble metallic state. Bielschowsky method is one of the most reliable ammoniacal silver impregnation methods and is particularly suited to diagnostic material.

**Specimen:** Paraffin-embedded tissue samples should be used.

**Procedure time:** 45 minutes.

**Prepare the following solutions immediately before use:**

- **Ammonia Water:** mix 8 drops of Reagent F in 50 mL of distilled water.
- **Ammoniacal Silver Solution:** Pour 25ml of **Reagent A** into container (using chemically cleaned glassware). Add **Reagent F**; drop by drop, while swirling the flask continuously, until precipitate just dissolves and the reagent goes clear.
- **Developer Solution:** mix 50 mL distilled water, 8 drops of **Reagent B**, 8 drops of **Reagent C** and 4 drops of **Reagent D**, swirl carefully throughout mixing steps.

### Staining procedure:

- 1) Deparaffinize and hydrate for paraffin section.
- 2) Put the crystal in distilled water.
- 3) Preheat waterbath to 40°C.
- 4) Place a chemically cleaned staining jar containing 25ml of **Reagent A** in waterbath and allow temperature to equilibrate for 10 minutes.
- 5) Place slide in warmed **Reagent A** and incubate for 15 minutes at 40°C.
- 6) During incubation place **Ammoniacal Silver Solution** in waterbath in allow temperature to equilibrate.
- 7) Remove slide from **Reagent A** and rinse in 4 changes of distilled water.
- 8) Place slide in warmed **Ammoniacal Silver Solution** and incubate for 10 minutes at 40°C.
- 9) 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).
- 10) Remove slide from **Developer Solution** and immediately place in **Ammonia Water** for 30 seconds.
- 11) Rinse in 4 changes of distilled water.
- 12) Apply adequate **Reagent E** to completely cover tissue section and incubate for 2 minutes.
- 13) Rinse in 4 changes of distilled water.
- 14) Dehydrate, clear in xylene and mount the preparation.

See our web site at [www.gennova-europe.com](http://www.gennova-europe.com) for detailed protocols ancillary reagents and support products.

## EXPECTED RESULTS:

During the reaction of the different solutions of the kit with the tissue sections, resulting in the following stains:



Catalog number



Batch code



In Vitro diagnostic medical device



Temperature limitation



Expiration date



Test number



Manufacturer



See instruction for use



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Axons	Black
Neurofibrillary tangles	Black
Senile plaques	Black
Nuclei	Dark brown
Background	Yellow to Light Brown

## **REQUIRED MATERIALS BUT NOT SUPPLIED:**

All reagents, materials, and laboratory equipment for this procedure are not provided with this kit. This includes adhesive slides and cover slips, positive and negative control tissues, xylene or adequate substitute, ethanol, distilled H<sub>2</sub>O, pipettes, Coplin jars, glass jars, moist chamber, histological baths, mounting materials, and microscope.

Buffered solutions for and other auxiliary reagents are available from Genova Scientific.

## **STORAGE AND STABILITY:**

After reception, storage the reagent according to temperature indicated on product label until the expiration date printed. Note that some reagents need to be storage 2-8 °C and other to room temperature. Do not use after the expiration date. Keep the containers tightly closed. After the first opening, the product is usable until the expiry date stated on label of intact product. Waste from solutions must be disposed under the procedure of hazardous substance. If the product is stored under different conditions from those stipulated in these technical indications, the new conditions must be verified by the user.

Genova Scientific guarantees that the product will maintain all of the described characteristics from the production date until the expiration date, as long as the product is stored and used as recommended. No other guarantees are provided. Under no circumstances is Genova Scientific obliged to cover damages caused by use of this reagent.

## **TROUBLESHOOTING:**

If unusual staining is observed or any other deviations from the expected results, please read these instructions carefully, and if this does not solve the problem, please contact Genova Scientific's technical support department or your local distributor.

## **PRECAUTIONS:**

Use only by qualified personnel. Read with attention the information written on the label (dangerous symbols, risks and safety phrases). Consult always the safety data sheet (MSDS) where the information about the risks of the preparation, precautionary measures during use, first aid and disposal are available. Use proper protective equipment in order to avoid contact with reagents and samples in the eyes, skin, and mucosal tissues. MSDS is available upon request.

## **PERFORMANCE CHARACTERISTICS:**

Genova Scientific has performed studies to evaluate the functioning of this product, concluding that the product is both specific and sensitive for recommended use.

## **BIBLIOGRAPHY:**

Peters A. A general purpose method of silver staining (part I impregnation, part II development, part III quantitative studies). 1955, Quart. J. micr. sci. Carleton's Histological Technique Fourth edition. Oxford, Drury & Wallington, 259-260.

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Catalog number



Batch code



In Vitro diagnostic medical device



Temperature limitation



Expiration date



Test number



Manufacturer



See instruction for use



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