BIOGNOST®

BIELSCHOWSKY KIT

IVD *In vitro* diagnostic medical device CE

Five-reagent kit for staining nerve cells

INSTRUCTIONS FOR USE

REF Product code: BLS-100T (for 100 tests)

Introduction

Bielschowsky kit is intended for visualizing nerve cells in histology (dendrites and axons) and in neuropathology for diagnosing pathology processes, such as Alzheimer's disease that creates distinctive structures known as senile plaques and neurofibrillary tangles. Silver impregnation method acc. to Bielschowsky enables nervous system' s neurons and axons staining; first through impregnation in silver nitrate solution, then through secondary impregnation in silver-ammonia solution, and finally through silver ion reduction - elementary silver precipitation on the section using developer containing formaldehyde. Sodium thiosulfate solution is used for rinsing and removing non-bound silver.

Product description

- **BIELSCHOWSKY KIT** - Five-reagent kit for staining nerve cells

The kit contains:	100 tests (BLS-100T)	Storage temperature:
Silver nitrate, 20% solution	30 mL (SN20-OT-30)	2-8°C
Ammonium water	100 mL (AV-OT-100)	15-25°C
Silver ammonia reagent stock solution	10 mL (SSAR-OT-10)	2-8°C
Developer stock solution	5 mL (STR-OT-5)	15-25°C
Sodium thiosulfate, 5% solution	100 mL (NT5-OT-100)	15-25°C

CAUTION:

Adhere to the following rules in order to achieve the best results:

- use distilled or demineralized high purity water **WITHOUT** any chlorine
- use completely clean laboratory glassware
- do not touch the sections / be in contact with metal objects (scissors, tweezers etc.) during staining
- let reagents reach room temperature before use (leave them at room temperature at least 2 hours before staining)
- apply the reagents so they completely cover the section

Preparing silver ammonia working solution:

Mix 2 mL of Silver ammonia reagent stock solution with 14 mL of demineralized water. Separate half of the prepared working solution (8 mL) and use in a later step (preparing the developer working solution).

NOTE: use silver ammonia working solution for a single staining; adjust its volume to number of sections. The prepared 8 mL is sufficient for about 20 sections if the working solution is dripped on sections.

Preparation of developer working solution:

Add 16 drops (about 750 μL) of Developer stock solution to 8 mL of previously prepared silver ammonia working solution.

NOTE: use developer working solution for a single staining and dispose of it after use; adjust its volume to number of sections. The prepared 8 mL is sufficient for about 20 sections.

Preparing the histological sections for staining

- Fix the sample (Formaldehyde NB 4%, Formaldehyde NB 10%, Bouin's solution), rinse with water and dehydrate through series of ascending alcohol solutions (Histanol 70, Histanol 80, Histanol 95 and Histanol 100).
- Clear the sample with intermedium; in xylene (BioClear) or in a xylene substitute (BioClear New).
- Infiltrate and fit the sample in paraffin (BioWax Plus, BioWax 56/58, BioWax Blue).
- Cut the paraffin block to **6-8** μm slices and place them on a VitroGnost adhesive glass slide.

Sample staining procedure

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 5 min each
2.	Rehydrate using 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate using 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled (demi) water	2 min
5.	Add Silver nitrate, 20% solution (≥5 kapi), incubate in dark place	20 min
6.	Add Silver ammonia working solution, (≥5 kapi), incubate in dark place	15 min
7.	Rinse with Ammonium water	drip and drain a few times
8.	Add Developer working solution, (≥5 kapi), incubate in dark place Note: macroscopically and microscopically observe the sections until reaching required coloration; sections get darker with longer treatment	2-3 minutes
9.	Rinse with Ammonium water	until the excessive reagent is washed off
10.	Rinse in distilled (demi) water	1 min
11.	Add Sodium thiosulfate, 5% solution (≥5 drops)	1 min
12.	Rinse under tap water	
13.	Dehydrate using 95% alcohol (Histanol 95)	2 min
14.	Dehydrate using 100% alcohol (Histanol 100)	2 exchanges, 2 min each
15.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 3 and 5 min

Immediately after clearing apply an appropriate BioMount medium for covering/mounting on the section. If BioClear xylene was used, use one of BioGnost's mounting xylene-based media (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate covering agent is BioMount New. Cover the section with VitroGnost cover glass.

Result

Nerve endings, neurofibrils, neurofibrillary tangles, neural plaques, nuclei - dark brown to black
Background - yellow to light brown

Note

Histology staining procedures are not standardized and they depend on standard operating procedures of individual laboratories and the experience of the personnel conducting the staining procedure. Intensity of staining depends on the period of immersion in the dye. Depending on personal requests and standard laboratory operating procedures, sample processing and staining can be carried out according to other protocols.

Preparing the sample and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples with modern technology and mark them clearly. Follow the manufacturer's instructions for handling. In order to avoid mistakes, the staining procedure and diagnostics should only be conducted by authorized and qualified personnel. Use only microscope according to standards of the medical diagnostic laboratory.

Safety at work and environmental protection

Handle the product in accordance with safety at work and environmental protection guidelines. Used solutions and out of date solutions should be disposed of as special waste in accordance with national guidelines. Chemicals used in this procedure could pose danger to human health. Tested tissue specimens are potentially infectious. Necessary safety measures for protecting human health should be taken in accordance with good laboratory practice. Act in accordance with signs and warnings notices printed on the product's label, as well as in BioGnost's material safety data sheet.

Storing, stability and expiry date

Bielschowsky kit's reagents have different storage temperature regime marked on their labels. Keep at declared temperatures, keep at cold place, do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's label.

References

1. Bielschowsky, M.(1908): Eine Modifikation meines Silverimprägnationsverfahrens zur Darstellung der Neurofibrillen. *J für Psychologie Neurologie* 12:135–137
2. Crookham, J., Dapson, R.(1991: Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, Anatech
3. Mirra, S.S., Hart, M.N., Terry, R.D.(1993): Making the Diagnosis of Alzheimer's Disease, *Arch Pathol Lab Med*. (117:132-144)

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	Refer to the supplied documentation	°C	Storage temperature range		Number of tests in package	REF	Product code	CE	European Conformity		BIOGNOST Ltd. Medjugorska 59 10040 Zagreb CROATIA www.biognost.com
	Refer to supplied instructions		Keep away from heat and sunlight		Valid until	LOT	Lot number		Manufacturer		
IVD	For *in vitro* diagnostic use only		Keep in dry place		Caution - fragile						