BIOGNOST®

BORAX, SOLUTION

IVD *In vitro* diagnostic medical device CE

For use with Grocott and PASM/Jones kits
INSTRUCTIONS FOR USE

REF Catalog number: BO-OT-100 (100 mL) BO-OT-250 (250 mL)

Introduction

Borax, solution is a component of Grocott and PASM/Jones kit used in histology for visualizing argentaffin structures, especially kidney membranes, but also fungi and certain pathogen organisms. Staining procedure starts with periodic acid solution being used to oxidize 1,2-glycols to aldehydes. During incubation in silver-methenamine-borate working solution aldehydes are reduced and at the same time cause reduction of silver ions to metallic silver that manifests as brown to black structures on the section. This is followed by toning the solution with gold chloride solution that additionally improves staining intensity of target structures (fungi, basal membranes and others), and it reduces background staining. Excessive unbound silver-gold bonds is removed by rinsing the section with sodium thiosulfate solution. The sections are exposed to counterstain that stains background structures; that in turn creates clear and visually rich contrast to target structures (colored brown-black).

Product description

BORAX, SOLUTION – Optimal concentration sodium tetraborate solution

Example of staining using Borax, solution as a component of PASM/Jones kit:

The kit contains:	**5 x 100 mL + 3 x 250 mL (PASM-K-100)**
Periodic acid, 1% solution	100 mL (PK1-OT-100)
Silver nitrate, solution	100 mL (SN-OT-100)
Methenamine, solution	250 mL (MET-OT-250)
Borax, solution	2x250mL (BO-OT-250)
Gold chloride, 0.2 solution	100 mL (ZK02-OT-100)
Sodium thiosulfate, 2% solution	100 mL (NT2-OT-100)
Nuclear Fast Red (Kernechtrot) reagent	100 mL (KR-OT-100)

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
- all the reagents must reach room temperature before use
- apply the reagents so they completely cover the section

Preparation of 120 ml of silver-methenamine-borate working solution (optimal for Schifferdecker jar):

Add 20 ml of Methenamine, solution and 22 ml of Borax, solution to 72 ml of double distilled (demi) water. Then gradually add 6 ml of Silver nitrate, solution by stirring using glass stick.

NOTE: silver-methenamine-borate working solution must be used for one staining only and be discarded after the use

Preparing the histological sections for staining

Fix the tissue sample tightly (4% NB Formaldehyde, 10% NB Formaldehyde), 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, BioWax Micro).
Cut the paraffin block to 4-6 μm slices and place them on a VitroGnost glass slide.

Sample staining procedure

1.	Prepare **silver-methenamine-borate working solution,** pour it into jar, cover with a glass lid and place in the water bath at 62°C	
	Note: we recommend using Coplin or Hellendahl jars	
2.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 2 min each
3.	Rehydrate using 100% alcohol (Histanol 100)	2 exchanges, 3 and 2 min
4.	Rehydrate using 95% alcohol (Histanol 95)	2 min
5.	Rehydrate in distilled (demi) water	2 min
6.	Immerse into Periodic acid, 1% solution	11 min
	Note: shorten incubation period to achieve fungi oxidation	5 min
7.	Rinse in double distilled (demi) water	6 exchanges, 5 seconds each
8.	Immerse the sections in previously heated methenamine-silver-borate working solution and incubate at 62°C. Check the section staining microscopically. If necessary, prolong the incubation period (if the fungi turn dark brown on light yellow background)	30 min
	Note: for staining fungi, incubate for 20 min and visually check until required staining intensity is achieved (fungi turn dark brown on light yellow background)	20 min for staining fungi
9.	Rinse in redistilled (demi) water (room temperature)	6 exchanges, 5 seconds each
10.	Immerse into Gold chloride, 0.2% solution	30 seconds
11.	Rinse in redistilled (demi) water (room temperature)	6 exchanges, 5 seconds each
12.	Immerse into Sodium thiosulfate, 2% solution	2 min
13.	Rinse well under tap water	2 min
14.	Immerse into Nuclear Fast Red (Kernechtrot) reagent	5-10 minutes
	Note: depending on personal preferences, instead of Nuclear Fast Red (Kernechtrot) reagent for counterstaining it is possible to stain by using Hematoxylin and Eosin solutions already routinely used in the laboratory	

15.	Rinse in distilled (demi) water	
16.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 30 seconds each
17.	Dehydrate using 100% alcohol (Histanol 100)	30 seconds
18.	Dehydrate using 100% alcohol (Histanol 100)	2 min
19.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 2 min each

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

Basal membranes, glycogen, bacteria and fungi - brown to black
Background - pink

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

Store Borax, solution in a tightly closed original packaging at temperature between 15°C and 25°C (as a kit component it can be stored at at temperature between 2°C and 8°C). Do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's label.

References

1. Bancroft, J.D., Gamble, M. Livingstone, C. Theory and practice of Histological Techniques – 5° edizione 2002.
2. Grocott. A Stain for fungi in tissue section and smears. Am J Pathol. 1955; 25:975.
3. Koski, J.P. (1981): Silver methenamine-borate (SMB): Cost reduction with technical improvement in silver nitrate-gold chloride impregnations. J. Histotechnol. 4; p 115.
4. Melis, M., Carpino, F., Di Tondo, U., Ermes, E. Techniche in anatomia patologica. 1989.

BO-OT-X, V1-EN1, 10 December 2018, IŠP/VR

	Refer to the supplied documentation	°C	Storage temperature range	Σ	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					CE	