BIOGNOST®

SODIUM THIOSULFATE, 2% SOLUTION

IVD *In vitro* diagnostic medical device CE

For use in special kits
INSTRUCTIONS FOR USE

REF Product code: NT2-OT-100 (100 mL)

Introduction

Sodium nitrate, 2% solution is a component of special staining kits, such as Grocott kit, stabilized, PASM/ Jones kit, stabilized, as well as Feulgen kit. Grocott kit and PASM/ Jones are used for visualizing argentaffin structures in histology. For diagnostic purposes it is most commonly used for impregnating basal membranes and fungi using silver. 1,2-glycols are oxidized to aldehydes by treating the section with periodic acid solution. During incubation in silver-methenamine-borate working solution aldehydes are reduced to primary alcohols with simultaneous reduction of silver ions to elementary silver (dark brown-black). Section toning follows using gold chloride solution that enhances target structures staining, as well as removes non-specific staining. Excessive unbound silver-gold bonds is removed by rinsing the section with sodium thiosulfate solution. Finally, the sections are exposed to Fast Green F.C.F. dye (Grocott kit, stabilized) or Nuclear Fast Red dye (PASM/ Jones kit, stabilized) that stains background structures green or red creating a clear and visually rich contrast to brown-black stained target structures.
Feulgen kit is used as cytochemical method of semiquantitative determining DNA in histology and cytology samples.

Product description

- **SODIUM THIOSULFATE, 2% SOLUTION** – Sodium thiosulfate aqueous solution

Example of using Silver sulfate, 2% solution in Grocott kit, stabilized

Other sections and reagents that may be used in staining:

- Fixatives such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydrating/rehydrating agent, such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95 and Histanol 100
- Clearing agents, such as BioClear xylene or a substitute, such as BioClear New agent on the aliphatic hydrocarbons basis
- Infiltration and fitting agent, such as BioGnost's granulated paraffin BioWax Plus, BioWax 52/54, BioWax 56/68, BioWax Blue.
- Covering agents for microscopic sections and mounting cover glass, such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount New Low, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount DPX Low Eco, BioMount C, BioMount Aqua, Canada Balsam
- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE, VitroGnost COLOR or one of more than 30 models of BioGnost's VitroGnost glass slides
- BioGnost's immersion media, such as Immersion oil, Immersion oil, types A, C, FF, 37, or Immersion oil Tropical Grade
- Other components of Grocott kit, stabilized: Periodic acid, 1% solution (PK1-OT-100), Methenamine, solution (MET-OT-100), Borax, solution (BO-OT-105), Gold chloride, 0.6% solution (ZK06-OT-100), Silver nitrate, stabilized (SNS-OT-100), Fast Green F.C.F. counterstaining reagent (FGKR-OT-100)

CAUTION:

- use distilled or demi high purity water **WITHOUT** any chlorine (electric conductivity $< 5.5\,\mu S$)
- use completely clean laboratory glassware
- do not touch sections or solutions with metal objects (metal spoons, tweezers and so on) during staining
- apply the reagents so they completely cover the section
- keep the reagents stored at temperature between +15 °C and +25°C. Precipitation in reagents and ineffective staining may occur at lower temperature

Preparation of methenamine-silver-borate working solution:

a) 40 ml volume (optimal for Coplin jar):

Add 15 ml of double distilled (demi) water, 3 mL of Methenamin, solution and 2 mL of Borax, solution into the jar. Then add 20 mL of Silver nitrate, stabilized solution and mix using glass stick.

b) 80 ml volume (optimal for Hellendahl jar):

Add 30 mL of double distilled (demi) water, 6 mL of Methenamine, solution and 4 mL of Borax, solution into the jar. Then add 40 mL of Silver nitrate, stabilized solution and mix 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 on 1-3 μm thin slides and mount them on VitroGnost glass slide; we recommend using adhesive slides (VitroGnost Plus Ultra, VitroGnost PLL, VitroGnost SIL, VitroGnost Super Frost Plus)

Sample staining procedure

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate using 100% alcohol (Histanol 100)	2 exchanges, 3 and 2 min
3.	Rehydrate using 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled (demi) water	2 min
5.	Add Periodic acid, 1% solution	5 min for fungi oxidation
	Note: prolong the incubation period for basal membrane oxidation	11 min
6.	Rinse in double distilled (demi) water	3 exchanges, 30 seconds each
7.	Prepare a fresh **silver-methenamin-borax working solution** and incubate with sections at +56°C in water bath. Check the section staining microscopically if necessary.	20-25 min for staining fungi
	Note: for staining basal membrane, incubate for 30 min and visually check until required staining intensity is achieved (basal membranes turn dark brown on light yellow background)	30-35 minutes
8.	Rinse in redistilled (demi) water (room temperature)	3 exchanges, 30 seconds each

9.	Add Gold chloride, 0.6% solution	30-60 seconds
	Note: longer exposition to Gold Chloride, 0.6% solution moves the membrane saturation hue from black to grey	
10.	Rinse in redistilled (demi) water (room temperature)	3 exchanges, 30 seconds each
11.	Add Sodium thiosulfate, 2% solution	2 min
12.	Rinse under indirect stream of tap water	2 min
13.	Add Fast Green F.C.F. counterstaining reagent	2-3 minutes
14.	Rinse in distilled (demi) water	
15.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 30 seconds each
16.	Dehydrate using 100% alcohol (Histanol 100)	30 seconds
17.	Dehydrate using 100% alcohol (Histanol 100)	2 min
18.	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 - green

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

Keep Sodium thiosulfate, 2% solution in a tightly sealed original packaging at temperature of 15 to 25°C. Keep in dry places, 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. Jones, D.B. (1957): Nephrotic Glomerulonephritis, Am J Pathol. Apr; 33(2): 313-329
4. Koski, J.P. (1981): Silver methenamine-borate (SMB): Cost reduction with technical improvement in silver nitrate-gold chloride impregnations. J. Histotechnol. 4; p 115.
5. Melis, M., Carpino, F., Di Tondo, U., Ermes, E. Techniche in anatomia patologica. 1989.

NT2-OT-100, V1-EN1, 16 January 2023 KB/IŠP

	Refer to the supplied documentation		Storage temperature range		Number of tests in package	REF	Product code	CE	European Conformity
	Refer to supplied instructions		Keep away from heat and sunlight		Do not re-use	LOT	Lot number		Manufacturer
IVD	For in vitro *diagnostic use only*		Keep in dry place		Caution - fragile		Valid until		

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