BIOGNOST®

GROCOTT KIT, STABILIZED

IVD *In vitro* diagnostic medical device CE

Seven-reagent kit for staining fungi and other argentaffin structures

INSTRUCTIONS FOR USE

REF Product code: GRC-100T (for 100 tests) GRC-K-100 (for 300-350 tests)

Introduction

Grocott kit is used in histology for visualization of argentaffin structures. For diagnostic purposes, it is mostly used for impregnating basal membranes and fungi using silver. The staining procedure is similar to the P.A.S. staining, however, the target structures are of more intense and stronger contrast after staining with the Grocott kit. The section is treated with the periodic acid solution used to oxidize 1,2-glycols to aldehydes. During incubation in methenamine-silver-borate working solution, aldehydes are reduced to primary alcohols with simultaneous reduction of silver ions to elementary silver (dark brown to black in color). This is followed by toning the section with gold chloride solution that additionally improves staining intensity of target structures, and it 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 that stains background structures green; that in turn creates clear and visually rich contrast to target structures (brown to black in color).
This is new and improved formulation for Grocott staining. Kit is stable at room temperature, do not store at lower temperature!

Product description

GROCOTT KIT, STABILIZED - Seven-reagent kit for staining fungi and other argentaffin structures

The kit contains:	100 tests (GRC-100T)	300-350 tests (GRC-K-100)
Periodic acid, 1% solution	30 mL (PK1-OT-30)	100 mL (PK1-OT-100)
Silver nitrate, stabilized solution	3 x 100 mL (SNS-OT-100)	2 x 500 mL (SNS-OT-500)
Methenamine, solution	50 mL (MET-OT-50)	2 x 100 mL (MET-OT-100)
Borax, solution	35 mL (BO-OT-35)	105 mL (BO-OT-105)
Gold chloride, 0.6% solution	30 mL (ZK06-OT-30)	100 mL (ZK06-OT-100)
Sodium thiosulfate, 2% solution	30 mL (NT2-OT-30)	100 mL (NT2-OT-100)
Fast Green F.C.F. contrast reagent	30 mL (FGKR-OT-30)	100 mL (FGKR-OT-100)

CAUTION:

- use distilled or demineralized high purity water **WITHOUT** any chlorine (< 5.5 µS of electrical conductivity)
- use completely clean laboratory glassware
- do not touch the sections or solutions with metal objects (scissors, tweezers, etc.) during staining
- apply the reagents so they completely cover the section
- keep the reagents at room temperature (between +15°C and +25°C). Lower temperatures may cause reagent precipitation and ineffective staining

Preparation of silver-methenamine-borate working solution:

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

Add 15 ml of double-distilled (demi) water, then add 3 ml of Methenamine, solution and 2 mL of Borax, solution. Then add 20 mL of Silver nitrate, solution and stir by using glass stick.

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

Add 30 ml of double-distilled (demi) water, then add 6 ml of Methenamine, solution and 4 mL of Borax, solution. Then add 40 mL of Silver nitrate, solution and stir by using glass stick.

NOTE: use silver-methenamine-borate working solution for single staining only and discard after 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 1-3 µm sections and place them on the VitroGnost glass slide. We recommend adhesive glass 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 **methenamine-silver-borate working solution** and incubate with the sections at +56°C in a water bath. Check the section staining microscopically if necessary.	20-25 mins 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 mins
8.	Rinse in double-distilled (demi) water (at room temperature)	3 exchanges, 30 seconds each
9.	Add Gold chloride, 0.6% solution	30-60 seconds
	Note: longer exposure period to Gold chloride, 0.6% solution shifts the membrane staining hue from black to gray	
10.	Rinse in double-distilled (demi) water (at room temperature)	3 exchanges, 30 seconds each
11.	Add Sodium thiosulfate, 2% solution	2 min
12.	Rinse well under indirect stream of tap water	2 min
13.	Add Fast Green F.C.F. contrast 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. The 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 a microscope according to the 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 warning notices printed on the product's label, as well as in BioGnost's material safety data sheet.

Storing, stability and expiry date

Keep Grocott kit in a tightly closed original package at temperatures between +15°C and +25°C. Keep in a dry place, do not freeze and avoid exposure 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; page 115.
5. Melis, M., Carpino, F., Di Tondo, U. Ermes, E. Techniche in anatomia patologica. 1989.

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