

GROCOTT KIT, STABILIZED

CE IVD *In vitro* diagnostic medical device

Classified acc. to Regulation (EU) 2017/746 - Class A device

Seven-reagent kit for staining fungi and other argentaffin structures

INSTRUCTIONS FOR USE

BASIC UDI number	385889212HPC30708STARVF		
EMDN code	W01030708		
REF	Catalog number	Volume	UDI-DI number
	GRC-100T	100 tests	03858890001747
	GRC-K-100	300-350 tests	03858890005615



Intended use and test principle

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 a 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)	Storage temperature
Periodic acid, 1% solution	30 mL (PK1-OT-30)	100 mL (PK1-OT-100)	15-25 °C
Silver nitrate, stabilized solution	3 x 100 mL (SNS-OT-100)	2 x 500 mL (SNS-OT-500)	15-25 °C
Methenamin, solution	50 mL (MET-OT-50)	2 x 100 mL (MET-OT-100)	15-25 °C
Borax, solution	35 mL (BO-OT-35)	105 mL (BO-OT-105)	15-25 °C
Gold chloride, 0.6% solution	30 mL (ZK06-OT-30)	100 mL (ZK06-OT-100)	15-25 °C
Sodium tiosulphate, 2% solution	30 mL (NT2-OT-30)	100 mL (NT2-OT-100)	15-25 °C
Fast Green F.C.F. contrast reageni	30 mL (FGKR-OT-30)	100 mL (FGKR-OT-100)	15-25 °C

Additional reagents and materials that can be used in this method

- 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 agent, such as BioClear xylene or its aliphatic hydrocarbon substitutes, such as BioClear New
- Infiltration and embedding agent, such as BioGnost's granulated paraffin BioWax Plus 56/58, 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 DPX, BioMount DPX High, BioMount DPX Low, BioMount C, BioMount Aqua
- VitroGnost slides and coverslips for use in histopathology and cytology
- BioGnost's immersion oils, such as Immersion oil, Cedarwood oil, Immersion oils types A and C, FF, 37 or Tropical Grade

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 (demineralized) 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 (demineralized) 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

Preparation of histological sections for staining

- Fix (Formaldehyde NB 4%, Formaldehyde NB 10%) and process the tissue sample
- Embed the tissue in a paraffin block (BioWax 52/54, BioWax 56/58, BioWax Plus 56/58, BioWax Blue)
- Cut the paraffin block into 4-6 µm thin slices and mount on a VitroGnost microscope slide

Sample staining procedure

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate in 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate in 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled/demineralized water	2 min
5.	Apply Periodic acid, 1% solution (≥ 5 drops)	5 min for fungi oxidation
	Note: prolong the incubation period for basal membrane oxidation	11 min

6.	Rinse in double-distilled/demineralized water	3 exchanges, 30 sec 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 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 min
8.	Rinse in double-distilled/demineralized water at room temperature	3 exchanges, 30 sec each
9.	Apply Gold chloride, 0.6% solution (≥ 5 drops)	30-60 sec
	Note: longer exposure period to Gold chloride, 0.6% solution shifts the membrane staining hue from black to gray	
10.	Rinse in double-distilled/demineralized water at room temperature	3 exchanges, 30 sec each
11.	Apply Sodium thiosulfate, 2% solution (≥ 5 drops)	2 min
12.	Rinse well under indirect stream of tap water	2 min
13.	Stain with Fast Green F.C.F. contrast reagent (apply ≥ 5 drops)	2-3 min
14.	Rinse in distilled/demineralized water	
15.	Dehydrate in 95% alcohol (Histanol 95)	2 exchanges, 30 sec each
16.	Dehydrate in 100% alcohol (Histanol 100)	30 sec
17.	Dehydrate in 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

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the staining procedure described in this Instruction for use may cause differences in staining results.

Sample preparation and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples using modern technology and mark them clearly. Be sure to follow the manufacturer's handling instructions. To avoid errors, staining and diagnosis can only be carried out by qualified personnel. Use a microscope equipped according to medical diagnostic laboratory standards. To avoid an incorrect staining result, it is advised to use a positive and negative control.

If a serious incident occurs during use of this product or as a result of its use, please report it to the manufacturer or authorized representative and competent authority.

Safety at work and environmental protection

Handle the product in accordance with occupational health and environmental protection guidelines. Used and expired solutions must be disposed of as special waste following national guidelines. Reagents used in this procedure can pose a danger to human health. The examined tissue samples are potentially infectious, and it is necessary to take the measures needed to protect human health in accordance with the guidelines of good laboratory practice. It is mandatory to read and act according to the information and warning signs printed on the product label and in the Safety Data Sheet, which is available on request.

Storage, stability, and shelf life

Upon receipt, store the product in a dry place and well-closed original packaging at a temperature of +15 °C to +25 °C. Do not freeze or expose to direct sunlight. After first opening, the product can be used until the specified expiry date, if stored properly. The expiration date is printed on the product label.

References

1. Bancroft, J.D., Gamble, M. Livingstone, C. *Theory and practice of Histological Techniques – 5^a 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; str. 115.
5. Melis, M., Carpino, F., Di Tondo, U., Ermes, E. *Techniche in anatomia patologica.* 1989.

Warnings and precautions regarding the materials contained in the product:

	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long – lasting effects.
	H60FD	May damage fertility. May damage the unborn child.
	P273	Avoid release to the environment.
	P280	Wear protective gloves/protective clothing/eye protection/face protection.
	P501	Dispose of contents in accordance to local regulations.
	P302+P352	IF ON SKIN: wash with plenty of water.
	P308+P313	IF exposed or concerned: call a POISON CENTER/doctor.

GRC-IFU_ENV13, 19.02.2026., IŠP

 Manufacturer	 Batch code	 Consult Instructions for use	 Contains sufficient for <n> tests
 Date of manufacture	 Catalogue number	 Caution	 European conformity
 Use-by date	 Temperature limit	 <i>In vitro</i> diagnostic medical device	 Unique device Identifier

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Version	Description / reason for change	Date
13	Revised in acc. to Regulation (EU) 2017/746 - IVDR	19.02.2026.