BIOGNOST®

GRIMELIUS KIT

IVD In vitro diagnostic medical device CE

Grimelius kit for staining argirophyl granules
INSTRUCTIONS FOR USE

REF Catalogue number: GRI-100T (for 100 tests) GRI-K-100 (4 x 100 mL + 15x3.5 g)

Introduction

Grimelius kit is used in histology for visualization of argirophyl structures in histology tissue sections. Certain tissues, such as neuroendocrine tumors can bind to silver ions from silver nitrate solution, but not reduce them to the visible form - elementary silver; this is why silver ion reduction during staining is achieved by exposing the section to the reducing hydroquinone solution and sodium sulfite. Excessive unbound silver ions are removed by rinsing the section with sodium thiosulfate solution.

Product description

GRIMELIUS KIT - Five-reagent kit for staining argirophyl granules.

The kit contains:	For 100 tests (GRI-100T)	4 x 100 mL + 15x3.5 g (GRI-K-100)
Silver nitrate, 1% solution	30 mL (SN1-OT-30)	100 mL (SN1-OT-100)
Acetate buffer, Grimelius	30 mL (APG-OT-30)	100 mL (APG-OT-100)
Hydroquinone - sodium sulphite	15 x 1.75 g (HNS-15-175)	15 x 3.5 g (HNS-15-35)
Sodium thiosulfate, 5% solution	30 mL (NT5-OT-30)	100 mL (NT5-OT-100)
Nuclear Fast Red (Kernechtrot) reagent	30 mL (KR-OT-30)	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

Silver nitrate working solution preparation with:

a) using kit for 100 tests (GRI-100T)

Gradually add 2 mL of Silver nitrate, 1% solution and 4 mL of Acetate buffer, Grimelius to 40 mL of double distilled (demi) water by mixing using glass stick. **Separate 1-2 mL of working solution for the second impregnation.**

b) using kit of bigger volume (GRI-K-100):

Gradually add 6 mL of Silver nitrate, 1% solution and 12 mL of Acetate buffer, Grimelius to 120 mL of double distilled (demi) water by mixing using glass stick. **Separate half the volume of working solution for the second impregnation.**

Preparation of reducing solution with:

a) using kit for 100 tests (GRI-100T)

Add 1 bag of Hydroquinone - sodium sulfite, powder in 50 mL of double distilled (demi) water by mixing using glass stick. **Separate 1-2 mL of reducing solution for the second impregnation.**

b) using kit of bigger volume (GRI-K-100):

Add 1 bag of Hydroquinone - sodium sulfite, powder in 100 mL of double distilled (demi) water by mixing using glass stick. **Separate half the volume of reducing solution for the second impregnation.**

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.

Procedure for staining the section using the kit for 100 tests (GRI-100T)

a) using kit for 100 tests (GRI-100T)

1.	Prepare **silver nitrate working solution,** pour it into jar, cover with a glass lid and place in the water bath at 60°C Separate 1-2 mL of working solution for the second impregnation.	
	Prepare **reducing solution,** pour it into jar, cover with a glass lid and place in the water bath at 60°C for 30 minutes before step 9. Separate 1-2 mL of reducing solution for the second impregnation.	
	Note: we recommend using Coplin or Hellendahl jars	
2.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 3 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
	FIRST IMPREGNATION:	
6.	Dip the sections in **silver nitrate working solution** and incubate in a dark place at temperature of 60°C.	3 hours
7.	Cool the sections down to room temperature	5 min
8.	Pour the reagent off the section without rinsing	
9.	Dip the sections in **reducing solution** and incubate in a dark place at temperature of 60°C.	5 min

10.	Cool the sections down to room temperature	5 min
11.	Rinse the section in distilled water	3 min
	SECOND IMPREGNATION:	
12.	Add **silver nitrate working solution** (≥5 drops) and keep at room temperature.	10 min
13.	Pour the reagent off the section without rinsing	
14.	Add **reducing solution** (≥5 drops) and keep at room temperature.	5 min
15.	Rinse the section in distilled water	3 min
16.	Add **Sodium thiosulfate, 5% solution** (≥5 drops)	2 min
17.	Rinse the section in distilled water	
18.	Add **Nuclear Fast Red (Kernechtrot) reagent** (>5 drops)	1-5 minutes
19.	Rinse the section in distilled water	
20.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
21.	Dehydrate using 100% alcohol (Histanol 100)	2 min
22.	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 a VitroGnost cover glass.

b) using kit of bigger volume (GRI-K-100):

1.	Prepare **silver nitrate working solution,** pour it into jar, cover with a glass lid and place in the water bath at 60°C Separate 1-2 mL of working solution for the second impregnation.	
	Prepare **reducing solution,** pour it into jar, cover with a glass lid and place in the water bath at 60°C for 30 minutes before step 9. Separate 1-2 mL of reducing solution for the second impregnation.	
2.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 3 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
	FIRST IMPREGNATION:	
6.	Dip the sections in **silver nitrate working solution** and incubate in a dark place at temperature of 60°C.	3 hours
7.	Cool the sections down to room temperature	5 min
8.	Pour the reagent off the section without rinsing	
9.	Immerse the sections into **reducing solution,** incubate at 60 °C	5 min
10.	Cool the sections down to room temperature	5 min
11.	Rinse the section in distilled water	3 min
	SECOND IMPREGNATION:	
12.	Dip the sections in the **silver nitrate working solution** and keep at room temperature.	10 min
13.	Pour the reagent off the section without rinsing	
14.	Dip the sections in the **reducing solution** and keep at room temperature.	5 min
15.	Rinse the section in distilled water	3 min
16.	Immerse in **Sodium thiosulfate, 5% solution**	2 min
17.	Rinse the section in distilled water	
18.	Immerse into **Nuclear Fast Red (Kernechtrot) reagent**	1-5 minutes
19.	Rinse the section in distilled water	
20	Dehydrate using 95% alcohol (Histanol 95)	5 dips
21.	Dehydrate using 100% alcohol (Histanol 100)	2 min
22.	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 a VitroGnost cover glass.

Results

Argirophyl granules: bright brown to black
Background: yellow-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

Keep Girmelius kit in a tightly sealed original packaging at temperature of 2°C to 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.

GRI-X, V2-EN2, 9 May 2017, AK/VR

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				