BIOGNOST®

A.F.O.G. KIT

IVD *In vitro* diagnostic medical device CE

Acid Fuchsin Orange G kit for staining glomerular protein deposits in kidney biopsies

INSTRUCTIONS FOR USE

REF Product code: AFOG-100T (for 100 tests) AFOG-K-100 (6x100 mL)

Introduction

A.F.O.G. kit is used for staining kidney biopsies. It can be used instead of P.A.S.M. kit because it consists of Aniline Blue, Orange G and Acid Fuchsin combination of dyes. If the kit is used with tissues fixed in formalin, muscle tissues are stained red instead of green.

Product description

- **A.F.O.G. KIT**- Six-reagent kit for staining and visualization of glomerular proteins in kidney biopsies.

The kit contains:	100 tests (AFOG-100T)	6 x 100 mL (AFOG-K-100)
Bouin's solution	100 mL (BOU-OT-100)	100 mL (BOU-OT-100)
Hematoxylin, Weigert A	30 ml (HEMA-OT-30)	100 ml (HEMA-OT-100)
Ferri reagent, Weigert B	30 ml (FR-OT-30)	100 ml (FR-OT-100)
A.F.O.G. reagent	30 mL (AFOG-OT-30)	100 mL (AFOG-OT-100)
Phosphomolybdic acid, 1% solution	30 mL (FMK1-OT-30)	100 mL (FMK1-OT-100)
HCL reagent, A.F.O.G	30 mL (HCLAF-OT-30)	100 mL (HCLAF-OT-100)

Other slides 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 56/58, BioWax 56/68, BioWax Blue, BioWax Micro
- Glass slides used in histology, pathology and cytology, such as VitroGnost SUPER GRADE or VitroGnost COLOR , or one of 30 (and more) BioGnost's glass slides
- 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 New, BioMount C, and Canada Balsam
- VitroGnost cover glass, dimensions range from 18x18 mm to 24x60 mm
- BioGnost's immersion media, such as Immersion oil, Immersion oil, types 37, A, C, FF, Tropical Grade, Cedarwood oil

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 56/58, 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.	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, 5 and 3 min
3.	Rehydrate using 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled (demi) water	2 min
5.	Stain using Bouin's solution	1-3 hours at 56˚C
6.	Cool the section down at room temperature	10 min
7.	Rinse in distilled water	10 seconds
8.	Stain using Hematoxylin, Weigert A (5 drops) and Ferri reagent, Weigert B (5 drops)	5 min
9.	Rinse under tap water	5 min
10.	Differentiate the section with A.F.O.G. HCL reagent Note: this step is not mandatory, use according to own demands	4-10 seconds
11.	Quickly rinse in distilled water	
12.	Treat with Phosphomolybdic acid, 1% solution	5 min
13.	Quickly rinse in distilled water	
14.	Stain with A.F.O.G. reagent	5-10 minutes
15.	Rinse in distilled water	1 min
16.	Dehydrate using 70% alcohol (Histanol 70)	5 dips
17.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
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 a VitroGnost cover glass.

Result

Nuclei - dark blue to black
Muscle fibers - green (red if the tissue is fixed with formalin)
Basal membrane - cyclamen color
Fibrin, erythrocytes - hues ranging from yellow to red
Connective fibers - blue

Note

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 A.F.O.G. kit 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. Melis, M., Carpino, F., Di Tondo, U. (1989), Tecniche in anatomia patologica, Edi Ermes, Milano.
2. Prophet, E.B., Mills, B., Arrington, J., Sobin, L. (1968), Laboratory methods in histotechnology, McGraw Hill, Washington D.C.
3. Bancroft, J.D., Gamble, M. (2002), Theory and practice of Histological Techniques, Churchill Livingstone, New York.

AFOG-X, V5-EN2, 10 January 2017, AK/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	