

A.F.O.G. KIT



IVD In vitro diagnostic medical device

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

Acid Fuchsin Orange G kit for staining kidney biopsies

INSTRUCTIONS FOR USE

Basic UDI-DI	385889212HPC30708STARVF		
EMDN code	W01030708		
REF	Catalog number	Volume	UDI-DI
AFOG-100T		100 tests	03858892125854
AFOG-K-100		6 x 100 mL	03858892125861



Intended use and test principle

The A.F.O.G. kit is intended for histological staining, primarily of kidney biopsies, for the differentiation of fibrin, connective tissue, and other tissue structures. The staining method is based on trichrome differential tissue staining using a mixture of three acidic dyes in the presence of phosphomolybdic acid. Phosphomolybdic acid first binds to selected tissue structures (particularly collagen and certain membranes) and acts as a "bridge" that increases the affinity of the amphoteric dye (Aniline Blue) for these components. After treatment with phosphomolybdic acid, the A.F.O.G. reagent is applied, which contains a combination of dyes: Aniline Blue, Orange G, and Acid Fuchsin. Each of these dyes exhibits selective affinity for specific tissue structures at low pH: Aniline Blue selectively stains collagen and connective tissue, which has been previously "activated" by phosphomolybdic acid; Orange G stains the cytoplasm and other tissue elements that do not have a strong affinity for phosphomolybdic acid; and finally, Acid Fuchsin selectively highlights glomerular protein deposits and immune complexes, thereby improving their visualization relative to the background tissue. The combination of these dyes provides a clear contrast between protein deposits, collagen, and cytoplasm, enabling reliable morphological assessment of glomerular lesions.

Product description

- **A.F.O.G. KIT** - A 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)	Storage temperature
Bouin's solution	100 mL (BOU-OT-100)	100 mL (BOU-OT-100)	15-25 °C
Hematoxylin, Weigert A	30 mL (HEMA-OT-30)	100 mL (HEMA-OT-100)	15-25 °C
Ferric reagent, Weigert B	30 mL (FR-OT-30)	100 mL (FR-OT-100)	15-25 °C
A.F.O.G. reagent	30 mL (AFOG-OT-30)	100 mL (AFOG-OT-100)	15-25 °C
Phosphomolybdic acid, 1% solution	30 mL (FMK1-OT-30)	100 mL (FMK1-OT-100)	15-25 °C
HCL reagent, A.F.O.G.	30 mL (HCLAF-OT-30)	100 mL (HCLAF-OT-100)	15-25 °C

Additional reagents and materials that can be used in the method

- Fixatives, such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydration/rehydration agents such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95, and Histanol 100
- Clearing agents, such as BioClear xylene or BioClear New, an aliphatic hydrocarbon-based xylene substitute
- Infiltration and embedding agents such as BioGnost's granulated paraffins BioWax 52/54, BioWax 56/58, BioWax Plus 56/58, BioWax Blue
- Microscopic slide covering agents and cover glass mountants 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, BioMount Aqua
- VitroGnost slides and coverslips for use in histopathology and cytology
- Immersion oils such as BioGnost's Immersion Oil, Immersion Oils types A, C, FF, 37, or Immersion Oil Tropical Grade

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 micron thin sections and mount on a VitroGnost microscope slide

NOTE

Apply the reagent so that it completely covers the section.

Sample staining procedure

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

1.	Deparaffinize in xylene (BioClear) or 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 minutes
4.	Rehydrate in distilled/demineralized water	2 min
5.	Immerse in Bouin's solution	1-3 hours at 56 °C
6.	Cool the section down to room temperature	10 min
7.	Rinse in distilled/demineralized water	10 s
8.	Add 5 drops of Hematoxylin, Weigert A and 5 drops of Ferric reagent, Weigert B. Mix gently and allow to react	5 min
9.	Rinse under running tap water	5 min
10.	Differentiate the section with HCL reagent, A.F.O.G. (add ≥ 5 drops) Note: this step is optional, apply according to your own requirements	4-10 s
11.	Rinse briefly in distilled/demineralized water	
12.	Apply Phosphomolybdic acid, 1% solution (≥ 5 drops)	5 min
13.	Rinse briefly in distilled/demineralized water	
14.	Apply A.F.O.G. reagent (≥ 5 drops)	5 - 10 min
15.	Rinse in distilled/demineralized water	1 min
16.	Dehydrate in 70% alcohol (Histanol 70)	5 dips
17.	Dehydrate in 95% alcohol (Histanol 95)	5 dips
18.	Dehydrate in 100% alcohol (Histanol 100)	2 min
19.	Clear in xylene (BioClear) or xylene substitute (BioClear New)	2 exchanges, 2 min each

Immediately after clearing, apply an appropriate BioMount covering/mounting medium. If BioClear xylene was used, use one of BioGnost's xylene-based mountants (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate mountant is BioMount New. Cover the slide with a VitroGnost cover glass.

b) using six-reagent kit of 100 mL (AFOG-K-100)

Pour the reagents into glass staining jars (type Coplin, Hellendahl, or Schifferdecker) and return them to the original bottles after staining. Close well. Filter the reagents as needed.

1.	Deparaffinize in xylene (BioClear) or 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.	Immerse in Bouin's solution	1-3 hours at 56 °C
6.	Cool the slide down to room temperature	10 min
8.	Rinse in distilled/demineralized water	10 s
9.	Prepare the Weigert hematoxylin working solution: mix an equal volume of Hematoxylin, Weigert A and Ferric reagent, Weigert B	
	Note: the working solution is stable for approximately two weeks. Prepare a volume of the working solution sufficient for staining the test sections	
10.	Immerse in Weigert hematoxylin working solution and allow to react	5 min
11.	Rinse under running tap water	3 min
12.	Differentiate the slide with HCL reagent, A.F.O.G. Note: this step is optional, apply according to your own requirements	4-10 s
13.	Rinse briefly in distilled/demineralized water	
14.	Immerse in Phosphomolybdic acid, 1% solution	5 min
15.	Rinse briefly in distilled/demineralized water	
16.	Immerse in A.F.O.G. reagent	5 - 10 min
17.	Rinse in distilled/demineralized water	1 min
18.	Dehydrate in 70% alcohol (Histanol 70)	5 dips
19.	Dehydrate in 95% alcohol (Histanol 95)	5 dips
20.	Dehydrate in 100% alcohol (Histanol 100)	2 min
21.	Clear in xylene (BioClear) or xylene substitute (BioClear New)	2 exchanges, 2 min each

Immediately after clearing, apply an appropriate BioMount covering/mounting medium. If BioClear xylene was used, use one of BioGnost's xylene-based mountants (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate mountant is BioMount New. Cover the slide with a VitroGnost cover glass.

Result

Nuclei - dark blue to black
Muscle fibers - green (red if the tissue is formalin-fixed)
Basement membrane - fuchsia/pink
Fibrin, erythrocytes - shades from yellow to red
Connective fibers - blue

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations in the staining procedure may cause differences in 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. It is necessary to follow the manufacturer's instructions for use. To avoid errors, staining and diagnosis may only be performed by qualified personnel. Use a microscope that complies with medical diagnostic laboratory standards. If a serious incident occurs during use or as a result of its use, please report it to the manufacturer and/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, therefore it is necessary to implement human health protection measures in accordance with good laboratory practice guidelines. It is mandatory to read and act according to the information and warning signs printed on the product label, instructions for use 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 production date and expiration date are printed on the product label.

References

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

Warnings and precautions regarding the materials contained in the product:

	H225	Highly flammable liquid and vapour.
	H302	Harmful if swallowed.
	H312	Harmful in contact with skin.
	H315	Causes skin irritation.
	H317	May cause an allergic skin reaction.
	H319	Causes serious eye irritation.
	H332	Harmful if inhaled.
	H335	May cause respiratory irritation.
	H341	Suspected of causing genetic defects.
	H350	May cause cancer.
	P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
	P233	Keep container tightly closed.
	P280	Wear protective gloves/protective clothing/eye protection/face protection.
	P301 + P330 + P331	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
	P302 + P352	IF ON SKIN: wash with plenty of water.
	P304 + P340	IF INHALED: remove person to fresh air and keep comfortable for breathing.
	P305 + P351 + P338	IF IN EYES: rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do Continue rinsing.
	P308 + P313	IF exposed or concerned: Get medical advice/attention.

AFOG-IFU_ENV3, 01.07.2026., IŠP

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

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Version	Description / reason for change	Date
3	Revised acc. to Regulation (EU) 2017/746 - IVDR	01.07.2026.