BIOGNOST®

PARALDEHYDE FUCHSIN KIT

IVD *In vitro* diagnostic medical device CE

Seven-reagent kit for detecting pathologic changes in elastic fibers acc. to Gomori

INSTRUCTIONS FOR USE

REF Catalogue number: PAF-100T (for 100 tests) PAF-K-100 (9 x 100 mL)

Introduction

Paraldehyde Fuchsin kit is used for visualization and detecting pathologic changes in elastic fibers, such as elastic tissue atrophy, loss or thinning of elastic tissue caused by atherosclerotic changes or vascular diseases. Often used for staining pancreatic beta cells, mastocyte granules, mucins, cartilage, argentaffin granules and acrosome of the sperm.

Product description

- **PARALDEHYDE FUCHSIN KIT** – Kit for staining elastic fibers, pancreatic cells, mastocytes, mucins and cartilage.

The kit contains:	100 tests (PAF-100T)	9 x 100 mL (PAF-K-100)
Potassium permanganate, 0.5% solution	30 mL (KP05-OT-30)	100 mL (KP05-OT-100)
Sulfuric acid, 0.5% solution	30 mL (SK05-OT-30)	100 mL (SK05-OT-100)
Oxalic acid, 1% solution	30 mL (OKS1-OT-30)	100 mL (OKS1-OT-100)
Histanol 70	2 x 30 mL (H70-30)	2 x 100 mL (H70-100)
Paraldehyde Fuchsin reagent	2 x 30 mL (PAF-OT-30)	2 x 100 mL (PAF-OT-100)
Nuclear Fast Red (Kernechtrot) reagent	30 mL (KR-OT-30)	100 mL (KR-OT-100)
Fast Green F.C.F. reagent	30 mL (FGR-OT-30)	100 mL (FGR-OT-100)

Other sections 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, BioWax 52/54, BioWax 56/68, BioWax Blue, BioWax Micro.
- 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 Low Eco, BioMount C, BioMount Aqua, Canada Balsam
- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE, VitroGnost COLOR or one of more than 30 models of BioGnost's VitroGnost glass slides
- VitroGnost cover glass, dimensions range from 18x18mm to 24x60mm
- BioGnost's immersion media, such as Immersion oil, Immersion oil, types A, C, FF, 37, or Immersion oil Tropical Grade

Preparing 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 52/54, 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.

NOTE

Apply the reagent so it completely covers the section.

Sample staining procedure

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

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.	Apply 5 drops of Potassium permanganate, 0.5% solution and 5 drops of Sulfuric acid, 0.5% solution. Gently stir on the section and let it react.	10 min
6.	Rinse in distilled (demi) water	
7.	Add Oxalic acid, 1% solution (≥5 drops)	5 min
8.	Rinse in distilled (demi) water	
9.	Treat with Histanol 70 (add ≥5 drops)	5 min
10.	Decant the section without rinsing and add Paraldehyde Fuchsin reagent (≥10 drops)	5-20 minutes
	Note: satisfying staining results are achieved after 5 minutes of exposure However, if you wish to achieve stronger and more intense coloration of the section, keep Paraldehyde Fuchsin reagent applied for 20 minutes on the section, but immerse the section into the incubation box to avoid reagent evaporation.	
11.	Decant the section and treat with Histanol 70 (add ≥5 drops)	10 min
12.	Rinse in distilled (demi) water	
13.	Stain with Nuclear Fast Red (Kernechtrot) reagent (add ≥5 drops)	10 min
14.	Rinse in distilled (demi) water	
15.	Stain with Fast Green F.C.F. reagent (add ≥5 drops)	5 min
16.	Rinse in distilled (demi) water	
17.	Dehydrate using 70% alcohol (Histanol 70)	5 dips

18.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
19.	Dehydrate using 100% alcohol (Histanol 100)	2 min
20.	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 seven-reagent 100 mL or 500 ml kit (PAF-K-100, PAF-K-500)

Pour the reagents into glass staining jars (Coplin, Hellendahl or Schifferdecker), return to original bottles after staining. Close tightly. Filter the reagents if necessary.

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.	Mix equal volumes of Potassium permanganate, 0.5% solution and Sulfuric acid, 0.5% solution. Gently stir and immerse the section.	10 min
6.	Rinse in distilled (demi) water	
7.	Immerse into Oxalic acid, 1% solution	5 min
8.	Rinse in distilled (demi) water	
9.	Immerse in Histanol 70	5 min
10.	Decant the section without rinsing, then immerse in Paraldehyde Fuchsin reagent	5-20 minutes
	Note: satisfying staining results are achieved after 5 minutes of exposure However, if you wish to achieve stronger and more intense coloration of the section, keep Paraldehyde Fuchsin reagent applied for 20 minutes on the section, but put the lid on the container to avoid reagent evaporation.	
11.	Decant the section and immerse in Histanol 70.	10 min
12.	Rinse in distilled (demi) water	
13.	Stain using Nuclear Fast Red (Kernechtrot) reagent	10 min
14.	Rinse in distilled (demi) water	
15.	Stain using Fast Green F.C.F. reagent	5 min
16.	Rinse in distilled (demi) water	
17.	Dehydrate using 70% alcohol (Histanol 70)	5 dips
18.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
19.	Dehydrate using 100% alcohol (Histanol 100)	2 min
20.	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

Pancreatic beta cells granules, elastic fiber, mastocytes, sulfated mucins - dark purple
Nuclei - red-purple
Connective tissue - green

Note

Time periods of staining processes are not entirely standardized and they approximately correspond to clinical and laboratory practical experience. Intensity of staining depends on the period of immersion in the dye. Real staining protocol depends on personal requests and priorities.

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 taken care of as a 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 Paraldehyde Fuchsin kit in a tightly sealed original packaging at temperature of 15 to 25°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. Culling, C.F.A.(1974): Handbook of histopathological and histochemical techniques, 2nd ed., Butterworth, London, UK.
2. Gomori, G. (1950): Aldehyde-Fuchsin: A New Stain for Elastic Tissue, Am. J. Clin. Path., 20; pp 665-666
3. Sheehan D.C. et Hrapchak, B.B.(1980): Theory an Practice Histotechnology, 2nd ed., CV Mosby, St. Louis, (MO), pp 52, p 14-167.

PAF-X, V6-EN3, 15 February 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				