BIOGNOST®

ACETIC ACID, 0.5% SOLUTION

IVD *In vitro* diagnostic medical device CE

For use with special kits

INSTRUCTIONS FOR USE

REF Product code: OK05-OT-100 (100 mL)

Introduction

Acetic acid, 0.5% solution can be used as a component of Movat special stain kit used for visualization of five types of connective tissues in a single staining process. It enables differentiation between collagens, muscle fibers, reticulin fibers, mucins and fibrins, and it also stains nuclei. It is used for diagnosing cardiovascular and pulmonary diseases. The function of Acetic acid, 0.5% solution is mild differentiation and removal of excessive dye from the section, which in turn makes microscopical image of the section better.

Product description

- **ACETIC ACID, 0.5% SOLUTION** - Acetic acid aqueous solution.

Example of staining histology samples with Acetic acid, 0.5% solution as a component of Movat kit

Other sections and reagents necessary for 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
- Movat kit components: Alcian Blue solution pH 2.5 (product code AB2-OT-30, AB2-OT-100), Alkaline alcohol, solution (product code ALA-OT-100), Hematoxylin, Verhoeff A (product code HEMV-OT-30, HEMV-OT-100), Ferri reagent, Verhoeff B (product code FRV-OT-30, FRV-OT-100), Iodine solution, Verhoeff C (product code JODV-OT-30, JODV-OT-100), Reagent for differentiation, Verhoeff (product code RDV-OT-30, RDV-OT-100), Sodium thiosulfate, 5% solution (product code NT5-OT-30, NT5-OT-100), Biebrich Scarlet-Acid Fuchsin reagent (product code BSAF-OT-30, BSAF-OT-100), Phosphotungstic acid, 5% solution (product code FVK5-OT-30, FVK5-OT-100), Orange G, 1% solution (product code ORG1-OT-30, ORG1-OT-100)

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 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.

Sample staining procedure

NOTE
Apply the reagent so it completely covers the section.

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 Alcian Blue solution pH 2.5 (add ≥5 drops)	20 min
6.	Rinse under tap water	5 min
7.	Add Alkaline alcohol, solution (≥5 drops and close in the chamber in order to prevent evaporation)	60 min
8.	Rinse under tap water	10 min
9.	Add 7 drops of Hematoxylin, Verhoeff A, 3 drops of Ferri reagent, Verhoeff B, and 3 drops of Iodine solution, Verhoeff C	15 min
10.	Rinse in distilled (demi) water	5 dips
11.	Add Reagent for differentiation, Verhoeff (≥5 drops) and differentiate the section	3 min
	Note: quickly rinse the section in distilled (demi) water after differentiation and microscopically check for the section for elastin being stained black. Repeat the differentiation if necessary	
12.	Rinse in distilled (demi) water	5 dips
13.	Differentiate in Acetic acid, 0.5% solution	5 dips
14.	Add Sodium thiosulfate, 5% solution (≥5 drops)	1 min
15.	Rinse under tap water	10 min
16.	Rinse in distilled (demi) water	5 dips
17.	Staining using Biebrich Scarlet-Acid Fuchsin reagent (≥5 drops)	3 min
18.	Differentiate in Acetic acid, 0.5% solution	5 dips
19.	Add Phosphotungstic acid, 5% solution	10 min
20.	Differentiate in Acetic acid, 0.5% solution	5 dips
21.	Rinse in distilled (demi) water	5 dips
22.	Stain using Orange G, 1% solution	15 min

23.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
24.	Dehydrate using 100% alcohol (Histanol 100)	2 min
25.	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
Nuclei and elastic fibers **-** black
Collagen and reticulin fibers - orange
Mucins - blue to green
Fibrins and muscle fibers - red

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 Acetic acid, 0.5% solution 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.

OK05-OT-100, V2-EN2, 16 May 2022, KB/IŠP

	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						