ACETIC ACID, 1% solution

IVD *In vitro* diagnostic medical device CE

1% acetic acid solution

INSTRUCTIONS FOR USE

REF Product code: OK1-OT-30 (30 mL) OK1-OT-100 (100 mL) OK1-OT-500 (500 mL)

Introduction

Masson-Goldner and Masson-Goldner Trichrome kits are used for visualization of muscles, collagen fibers and connective tissues, gametes, nuclei, neurofibrils, neuroglia, collagen, keratin intracellular fibrils and negative visualization of the Golgi apparatus. Masson Trichrome staining method utilizes Aniline Blue dye that binds to collagen, making it distinctively blue, while Masson-Goldner Trichrome utilizes Fast Green F.C.F. dye, making collagen green. The kits are also used for visualization of increased collagen build up associated with functioning tissue being mistaken for scar tissue (liver sclerosis diagnosis), but also for differentiating smooth muscle fibers and collagens. Acetic acid, 1% solution is a part of the mentioned kits. Its function is differentiation and removal of excessive dye from the section, which in turn makes microscopical image of the section better.

Product description

- **ACETIC ACID, 1% SOLUTION** - Acetic acid aqueous solution.

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

Example of staining histology samples using Masson Trichrome kit (MST-100T):

NOTE

Apply the reagent so it completely covers the section.
In order to avoid regaent evaporation from the section, we recommend using incubation chamber/plate.

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

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.	Staining using Bouin's solution: pour Bouin's solution in incubation jar (Coplin or Hellendahl) and immerse the section and cover it in order to avoid reagent evaporation. Leave at room temperature overnight or 60 min at +56°C.	60 min at 56˚C or over night at room temperature
6.	Cool the section down at room temperature	10 min
7.	Rinse under tap water	10 seconds
8.	Rinse in distilled water	10 seconds
9.	Apply 5 drops of Hematoxylin, Weigert A and 5 drops of Ferri reagent, Weigert B. Gently stir and let it react.	5 min
10.	Rinse under tap water	3 min
11.	Add Biebrich Scarlet-Acid Fuchsin reagent (≤5 drops)	2 min
12.	Rinse in distilled water	until the excessive dye is washed off of the section
13.	Add PTA-PMA reagent (≤5 drops)	10 min
14.	Pour the reagent off the section without rinsing	
15.	Add Aniline Blue reagent (≤5 drops)	5 min
16.	Rinse in distilled water	until the excessive dye is washed off of the section
17.	Add Acetic acid, 1% solution (≤5 drops)	3 min
18.	Dehydrate using 70% alcohol (Histanol 70)	5 dips
19.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
20.	Dehydrate using 100% alcohol (Histanol 100)	2 min
21.	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 - black
Muscle fibers, keratin, cytoplasm - bright red
Collagen, mucus - blue
Erythrocytes - red-orange

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, 1% solution in a tightly sealed original packaging at temperature of 15 to 25°C.s 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.

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!	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
i	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						