

ACID ALCOHOL



IVD *In vitro* diagnostic medical device

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

Reagent for differentiation during regressive hematoxylin staining method

INSTRUCTIONS FOR USE

BASIC UDI broj	385889212HPC30799PROCYU		
REF	Catalog number	Volume	UDI-DI broj
KA-OT-500		500 mL	03858892124864
KA-OT-1L		1000 mL	03858888820282
KA-OT-2.5L		2500 mL	03858890007480



Intended use and test principle

Acid alcohol is a differentiating reagent used in histology. It is applied in various staining methods, most commonly in regressive hematoxylin and eosin (HE) staining, where it plays the role of differentiating nuclei and non-nuclear structures. Due to the greater affinity of the dye for the nucleus, Acid alcohol will decolorize the cytoplasm. This occurs because the nuclear dye (aluminum-hematoxylin complex) binds more strongly to the nucleus than to the cytoplasm. Regardless of the type of fixative used to fix the tissue, Acid alcohol acts at almost the same speed and effect on different tissue samples.

Product description

- **ACID ALCOHOL** – Acid alcohol solution used for differentiation during regressive staining, it contains hydrochloric acid

An example of Acid alcohol use during regressive Hematoxylin and eosin (HE) staining procedure

Additional reagents and materials that can be used in this method

- 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 agent, such as BioClear xylene or its aliphatic hydrocarbon substitutes, such as BioClear New
- Infiltration and embedding agent, such as BioGnost's granulated paraffin BioWax Plus 56/58, BioWax 56/68, BioWax Blue
- Covering agents for microscopic sections and mounting cover glass, such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount C, BioMount Aqua
- VitroGnost slides and coverslips for use in histopathology and cytology
- BioGnost's immersion oils, such as Immersion oil, Cedarwood oil, Immersion oils types A and C, FF, 37 or Tropical Grade
- Nuclei bluing reagents such as BioGnost's hematoxylin solutions: Hematoxylin H or Hematoxylin G3
- Cytoplasm staining reagents such as BioGnost's eosin solutions
- Bluing reagents such as BioGnost's Bluing reagent or Scott's solution

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 μm thin slices and mount on a VitroGnost microscope slide

Hematoxylin and eosin (HE) manual staining procedure, regressive

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.	Stain using Hematoxylin H or Hematoxylin G3	4-8 min
	Note: In the case of subsidence in the solution or a formation of metallic glow on the surface, reagent should be filtered before use	
6.	Immerse the slide in distilled/demineralized water until dye is no longer being released from the slide	
7.	Differentiate using Acid alcohol	3-10 dips
	Note: This step removes excessive hematoxylin from the nucleus and cytoplasm. Discoloration of the nuclei can occur if the section is treated with the differentiation agent for too long	
8.	Rinse in distilled/demineralized water	
9.	Make nuclei turn blue using Scott's solution or Bluing reagent	1 min
	Note: Finish the process of bluing after the nuclei turn blue. If no Scott's solution or Bluing reagent is available, rinse the sections under tap water for 3-5 minutes	
10.	Immerse in distilled/demineralized water	
11.	If staining with an alcoholic eosin solution, immerse the sample into 95% alcohol (Histanol 95). If staining with a water – based eosin solution, skip this step	
12.	Stain using one of eosin contrast solutions until optimal staining is achieved	15 sec to 2 min
	Note: when staining with alcoholic eosin solutions strong eosinophilic staining is achieved (within 15 seconds). When staining with eosin water solutions, recommended time is 90 seconds to 2 minutes.	
13.	Rinse under tap water Note: If an alcoholic eosin solution is used for counterstaining, skip this step	
14.	Dehydrate in 95% alcohol (Histanol 95)	2 exchanges, 10-15 dips
15.	Dehydrate in 100% alcohol (Histanol 100)	3 exchanges, 10-15 dips
16.	Clear in xylene (BioClear) or 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 - blue

Cytoplasm, collagen, muscle fibers, erythrocytes – hues of pink

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the staining procedure described in this Instruction for use may cause differences in staining 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. Be sure to follow the manufacturer's handling instructions. To avoid errors, staining and diagnosis can only be carried out by qualified personnel. Use a microscope equipped according to medical diagnostic laboratory standards.

If a serious incident occurs during use of this product or as a result of its use, please report it to the manufacturer 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, and it is necessary to take the measures needed to protect human health in accordance with the guidelines of good laboratory practice. It is mandatory to read and act according to the information and warning signs printed on the product label 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 expiration date is printed on the product label.

References

1. Conn, J. (1977): Biological Stains, 9th ed. Baltimore: Williams and Wilkens Co.
2. Llewellyn, B.D. (2009): Nuclear staining with alum hematoxylin, Biotechnic and Histochemistry, 85(1), str. 159-177.
3. Sheehan, D.C. i Hrapchak, B.B. (1980): Theory and Practice of Histotechnology, 2. izd. St. Louise: CV Mosby Co.
4. Kiernan J. A. (2008) Histological and histochemical methods, 4th ed. Bloxham: Scion Publishing Ltd.

Warnings and precautions regarding the materials contained in the product:	
	<p>H226 Flammable liquid and vapor.</p> <p>P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.</p> <p>P233 Keep container tightly closed.</p> <p>P280 Wear protective gloves/protective clothing/eye protection/face protection.</p>

KA-IFU, ENV5, 20.02.2026., IŠP

 Manufacturer	 Batch code	 Consult instructions for use	 European conformity
 Date of manufacture	 Catalogue number	 Caution	 Unique device identifier
 Use-by date	 Temperature limit	 <i>In vitro</i> diagnostic medical device	

 **BioGnost Ltd.**
Medjugorska 59, 10040 Zagreb, Croatia, EU, www.biognost.com

Version	Description / reason for change	Date
5	Staining procedure added, QR code added, table with symbols revised, revised in acc. to Regulation (EU) 2017/746 - IVDR	20.02.2026.