BIOGNOST®

ALCIAN BLUE pH 2.5 KIT

CE IVD *In vitro* diagnostic medical device Classified acc. to Regulation (EU) 2017/746 - **Class A** device

Three-reagent kit for staining mucins acc. to Dorling

INSTRUCTIONS FOR USE

BASIC UDI number	385889212HPC30708STARVF	
EMDN code	W01030708	
REF **Catalog number**	**Volume**	**UDI-DI number**
AB25-100T	Za 100 testiranja	03858890000504
AB25-K-100	3x100 mL	03858890001389

Intended use and test principle

Histology, cytology and other related scientific disciplines study the microscopic anatomy of tissues and cells. In order to achieve a good tissue and cellular structure, the samples need to be stained in a correct manner. Alcian Blue dye is used to prove glycosaminoglycan in mucins, for staining amyloids, cysteines and for polychromatic staining of mastocytes according to Alcian-Blue Safranin. It is also used to determine bacterial species and detecting bacterial capsules. Alcian Blue pH 2.5 kit enables adequate staining and visualization of acid mucins without staining sulfated mucins. Rinsing solutions are of the same pH value, making the specificity of the reaction more intense. Cellular nuclei are stained red using a counterstain.

Product description

- **ALCIAN BLUE pH 2.5 KIT** – Three-reagent kit for staining mucins according to Dorling

The kit contains:	**100 tests (AB25-100T)**	**3 x 100 mL (AB25-K-100)**	**Storage temperature**
Alcian Blue solution pH 2,5	30 mL (AB2-OT-30)	100 mL (AB2-OT-100)	15-25°C
Sodium tetraborate, solution	30 mL (NTB-OT-30)	100 mL (NTB-OT-100)	15-25°C
Nuclear Fast Red (Kernechtrot) reagent	30 mL (KR-OT-30)	100 mL (KR-OT-100)	15-25°C

Additional reagents and materials that can be used in staining:

- Fixative agents 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
- BioGnost's immersion oils, such as Immersion oil, Cedarwood oil, Immersion oils types A and C, FF, 37 or 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 μm thin slices and mount on a VitroGnost microscope slide

NOTE

Apply the reagent so it completely covers the section.

Sample staining procedure

a) using kit for 100 tests

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 with Alcian Blue solution pH 2.5 (add ≥5 drops)	45 min
6.	Tilt the section and remove Alcian Blue solution pH 2.5. Without rinsing, cover the section with Sodium tetraborate, solution (add ≥5 drops)	10 min
7.	Rinse in distilled/demineralized water	5 min
8.	Stain with Nuclear Fast Red (Kernechtrot) reagent (add ≥5 drops)	5 min
9.	Rinse in distilled/demineralized water	2 min
10.	Dehydrate in 70% alcohol (Histanol 70)	5 dips
11.	Dehydrate in 95% alcohol (Histanol 95)	5 dips
12.	Dehydrate in 100% alcohol (Histanol 100)	2 min
13.	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 a VitroGnost cover glass

b) using three-reagent 100 mL kit (AB25-K-100)

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 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 into Alcian Blue solution pH 2.5	45 min
6.	Without rinsing, immerse into Sodium teraborate, solution	10 min

7.	Rinse in distilled/demineralized water	5 min
8.	Immerse into Nuclear Fast Red (Kernechtrot) reagent	5 min
9.	Rinse in distilled/demineralized water	2 min
10.	Dehydrate in 70% alcohol (Histanol 70)	5 dips
11.	Dehydrate in 95% alcohol (Histanol 95)	5 dips
12.	Dehydrate in 100% alcohol (Histanol 100)	2 min
13.	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 a VitroGnost cover glass.

Result

Nuclei – red
Mucins – blue

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, mounting of the slides, and diagnosis can only be carried out by qualified personnel. Use a microscope equipped according to medical diagnostic laboratory standards. To avoid an incorrect staining result, it is advised to use a positive and negative control.

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.
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.

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.

Literature

1. Conn, J. (1977): Biological Stains, 9th ed., Baltimore: Williams and Wilkins Co.
2. Mowry, R.W. (1956): Alcian blue techniques for the histochemical study of acidic carbohydrates, Journal of Histochemistry and Cytochemistry, 4, 407.
3. Scott, J.E., Dorling, J. (1965): Differential staining of acid glycosaminoglycans (mucopolysaccharides) by Alcian blue in salt solutions, Histochemie, 5, 221-233.

Warnings and precautions regarding the materials contained in the product:

H225	Highly flammable liquid and vapor.
H319	Causes serious eye irritation.
H336	May cause drowsiness and diziness.
P210	Keep away from heat, hot surface, sparks, open flames and pther ignition sources. No smoking.
P280	Wear protective gloves/protective clothing/ eye protection/face protection
P303 + P361 + P353	IF ON SKIN (or hair): take off immediately all contaminated clothing, rinse skin with water (or shower)
P305 + P351 + P338	IF IN EYES: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

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	Manufacturer	LOT	Batch code		Temperature limit	IVD	*In vitro* diagnostic medical device	UDI	Unique device identifier
	Date of manufacture	REF	Catalogue number		Consult instructions for use	Σ	Contains sufficient for <n>tests		
	Use-by date		Fragile, handle with care		Caution	CE	European conformity		

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Version	Description / reason for change	Date
5.	Revised in acc. to Regulation (EU) 2017/746 - IVDR	18.02.2026.