

# ALCIAN BLUE pH 2.5 KIT

IVD *In vitro* diagnostic medical device



## Three-reagent kit for staining mucins acc. to Dorling INSTRUCTIONS FOR USE

REF Product code: AB25-100T (for 100 tests) AB25-K-100 (3 x 100 mL)

### Introduction

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)
Alcian Blue solution pH 2.5	30 mL (AB2-OT-30)	100 mL (AB2-OT-100)
Sodium tetraborate, solution	30 mL (NTB-OT-30)	100 mL (NTB-OT-100)
Nuclear Fast Red (Kernechtrot) reagent	30 mL (KR-OT-30)	100 mL (KR-OT-100)

### Other slides 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 agent, such as BioClear xylene or its aliphatic hydrocarbon substitutes, such as BioClear New
- Infiltration and fitting agent, such as BioGnost's granulated paraffin BioWax Plus 56/58, BioWax 56/68, BioWax Blue, BioWax Micro
- Glass slides used in histology, pathology and cytology, such as VitroGnost SUPER GRADE or VitroGnost COLOR, or one of 30 (and more) BioGnost's glass slides
- 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, Canada Balsam or MountQuick Tube medium
- VitroGnost cover glass, dimensions range from 18x18 mm to 24x60 mm
- BioGnost's immersion oils, such as Immersion oil, Cedarwood oil, Immersion oils types A and C

### 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 Plus 56/58, BioWax 56/58, BioWax Blue, BioWax Micro)
- Cut the paraffin block to 4-6  $\mu$ m 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 (AB25-100T)

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.	Stain using Alcian Blue solution pH 2.5 (add $\geq$ 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 $\geq$ 5 drops)	10 min
7.	Rinse in distilled (demi) water	5 min
8.	Stain with Nuclear Fast Red (Kernechtrot) reagent (add $\geq$ 5 drops)	5 min
9.	Rinse in distilled (demi) water	2 min
10.	Dehydration using 70% alcohol (Histanol 70)	5 dips
11.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
12.	Dehydrate using 100% alcohol (Histanol 100)	2 min
13.	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 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 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.	Immerse into Alcian Blue solution pH 2.5	45 min
6.	Without rinsing, immerse into Sodium tartrate, solution	10 min
7.	Rinse in distilled (demi) water	5 min
8.	Immerse into Nuclear Fast Red (Kernechtrot) reagent	5 min
9.	Rinse in distilled (demi) water	2 min
10.	Dehydration using 70% alcohol (Histanol 70)	5 dips
11.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
12.	Dehydrate using 100% alcohol (Histanol 100)	2 min
13.	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

Nuclei - red  
Mucins - blue

### 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. In order to avoid an erroneous result, a positive and negative check is advised before application.

### 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 Alcian Blue pH 2.5 kit in a tightly closed original package at temperature between 15°C and 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. Conn, J. (1977): Biological Stains, 9<sup>th</sup> 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.

AB25-X, V5-EN4, 10 January 2017, AK/VR

 Refer to the supplied documentation	 Storage temperature range	 Number of tests in package	 Product code	 European Conformity
 Refer to supplied instructions	 Keep away from heat and sunlight	 Valid until	 Lot number	 Manufacturer
 For <i>in vitro</i> diagnostic use only	 Keep in dry place	 Caution - fragile		

 BIOGNOST Ltd.  
Medjugorska 59  
10040 Zagreb  
CROATIA  
www.biognost.com

