

# ALCIAN BLUE-P.A.S. KIT

IVD *In vitro* diagnostic medical device

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

## Alcian Blue – Periodic Acid-Schiff acid mucopolysaccharides staining kit acc. to Mowry

### INSTRUCTION FOR USE



<b>BASIC UDI number</b>	385889212HPC30708STARVF		
<b>EMDN code</b>	W01030708		
<b>REF</b>	<b>Catalogue number</b>	<b>Volume</b>	<b>UDI-DI number</b>
AB-100T		100 tests	03858890000528
AB-K-100		7 x 100 mL	03858888822347
AB-K-500		7 x 500 mL	03858890005691

#### Intended use and test principle

One of the most commonly used chemical methods in histology is PAS staining. Combined with Alcian Blue pH 2.5 solution on one slide it is possible to differentiate between neutral and acidic mucins, glycogen, and glycoproteins. Alcian Blue dye stains acidic mucins that become insoluble and resistant to the remaining reagents of the PAS staining procedure. The oxidizing action of periodic acid enables the characteristic purple (magenta) color in combination with the BioSchiff reagent. Nuclei are stained with Hematoxylin ML (Mayer-Lillie), which does not interfere with Alcian Blue dye.

#### Description of the product

- ALCIAN BLUE-P.A.S. KIT – Kit for staining neutral and acidic mucins and glycogen and glycoproteins

The kit contains:	100 tests (AB-100T)	7 x 100 mL (AB-K-100)	7 x 500 mL (AB-K-500)	Storage temperature
Alcian Blue solution pH 2.5	30 mL (AB2-OT-30)	100 mL (AB2-OT-100)	500 mL (AB2-OT-500)	15-25 °C
Sodium tetraborate, solution	30 mL (NTB-OT-30)	100 mL (NTB-OT-100)	500 mL (NTB-OT-500)	15-25 °C
Periodic acid, 0.8% solution	30 mL (PK08-OT-30)	100 mL (PK08-OT-100)	500 mL (PK08-OT-500)	15-25 °C
BioSchiff reagent	30 mL (BS-OT-30)	100 mL (BS-OT-100)	500 mL (BS-OT-500)	15-25 °C until opening, 2-8°C after opening
Sodium metabisulfite, solution	30 mL (NM-OT-30)	100 mL (NM-OT-100)	500 mL (NM-OT-500)	15-25 °C
HCL reagent, P.A.S.	30 mL (HCLP-OT-30)	100 mL (HCLP-OT-100)	500 mL (HCLP-OT-500)	15-25 °C
Hematoxylin ML	30 mL (HEMML-OT-30)	100 mL (HEMML-OT-100)	500 mL (HEMML-OT-500)	15-25 °C

#### Additional reagents and materials that can be used in the method

- 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
- Immersion media such as BioGnost's Immersion Oil, Immersion Oils types A, C, FF, 37, or Immersion Oil 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 micron thin slices and mount on a VitroGnost microscope slide

#### Preparation of the additional solution needed in the staining process

- Sulfite solution  
Note: make the sulfite solution directly **before** use!  
a) volume sufficient for 1 slide:  
In a small test tube, mix: 1 drop of Sodium metabisulfite, solution, 1 drop of HCL reagent, P.A.S. and 1 mL of tap water. Stir.  
b) volume sufficient for Hellendahl container (approximately 80 mL):  
Mix 3.75 mL of Sodium metabisulfite solution with 3.75 mL of HCL reagent, P.A.S. Then add another 75 mL of tap water to the solution, mix.

#### NOTE

Apply the reagent to completely cover the section.

#### Sample staining procedure

##### a) using kit for 100 tests (AB-100T)

1.	Deparaffinize in xylene (BioClear) or xylene substitute (BioClear New)	3 changes, 2 minutes each
2.	Rehydrate in 100% alcohol (Histanol 100)	2 changes, lasting 5 and 3 minutes
3.	Rehydrate in 95% alcohol (Histanol 95)	2 minutes
4.	Rehydrate in distilled/demineralized water	2 minutes
5.	Staining with Alcian Blue solution pH 2.5	30 minutes
6.	Tilt the slide and remove Alcian Blue solution pH 2.5. Without rinsing, cover the slide with sodium tetraborate solution	10 minutes
7.	Rinse under running tap water	5 minutes
8.	Rinse in distilled/demineralized water	1-2 minutes
9.	Treat with Periodic acid, 0.8% solution	5-10 minutes
10.	Rinse under running tap water	3 minutes
11.	Rinse the slide with distilled/demineralized water	
12.	Treat with BioSchiff reagent	10-15 minutes
13.	Treat with sulfite solution	3 changes, 2 minutes each
14.	Rinse under running tap water	3 minutes
15.	Staining with Hematoxylin ML	1-3 minutes
16.	Rinse under running tap water	3 minutes
17.	Dehydrate in 70% alcohol (Histanol 70)	5 dips
18.	Dehydrate in 95% alcohol (Histanol 95)	5 dips
19.	Dehydrate in 100% alcohol (Histanol 100)	2 minutes
20.	Clear in xylene (BioClear) or xylene substitute (BioClear New)	2 exchanges, 2 minutes each

Immediately after clearing, apply an appropriate BioMount covering/mounting medium. If BioClear xylene was used, use one of BioGnost's xylene-based mountants (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate mountant is BioMount New. Cover the section with a VitroGnost cover glass.

**b) by using kit with seven reagents of 100 or 500 mL (AB-K-100, AB-K-500)**

Pour the reagents into the staining containers (Coplin, Hellendahl or Schifferdecker type) and return them to the original bottles after staining. Close well. If necessary, filter the reagents.

1.	Deparaffinize in xylene (BioClear) or xylene substitute (BioClear New)	3 changes, 2 minutes each
2.	Rehydrate in 100% alcohol (Histanol 100)	2 changes, lasting 5 and 3 minutes
3.	Rehydrate in 95% alcohol (Histanol 95)	2 minutes
4.	Rehydrate in distilled/demineralized water	2 minutes
5.	Immerse in Alcian Blue solution pH 2.5	30 minutes
6.	Without rinsing, immerse in Sodium tetraborate solution	10 minutes
7.	Rinse under running tap water	5 minutes
8.	Rinse in distilled/demineralized water	1-2 minutes
9.	Immerse in Periodic acid, 0.8% solution	5-10 minutes
10.	Rinse under running tap water	3 minutes
11.	Rinse the slide with distilled/demineralized water	
12.	Immerse in BioSchiff reagent	10-15 minutes
Note: during staining, it is necessary to cover the container so that SO does not evaporate;		
13.	Immerse in sulfite solution without rinsing	3 changes, 2 minutes each
14.	Rinse under running tap water	3 minutes
15.	Immerse in Hematoxylin ML	1-3 minutes
16.	Rinse under running tap water	3 minutes
17.	Dehydrate in 70% alcohol (Histanol 70)	5 dips
18.	Dehydrate in 95% alcohol (Histanol 95)	5 dips
19.	Dehydrate in 100% alcohol (Histanol 100)	2 minutes
20.	Clear in xylene (BioClear) or xylene substitute (BioClear New)	2 exchanges, 2 minutes each

Immediately after clearing, apply an appropriate BioMount covering/mounting medium. If BioClear xylene was used, use one of BioGnost's xylene-based mountants (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate mountant is BioMount New. Cover the section with a VitroGnost cover glass.

**Result**

Mucins – blue turquoise color  
 P.A.S. positive substances – purple (magenta) color  
 Nuclei – blue color  
 Epithelial mucin and cartilage – purple/dark blue color

**Limitations**

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the described staining procedure may cause variations in 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. It is necessary to follow the manufacturer's instructions for use. To avoid errors, histological processing of samples and diagnosis may only be performed by qualified personnel. Use a microscope that complies with medical diagnostic laboratory standards. To avoid a false result, it is recommended to use a positive and negative control.

If a serious incident occurs during use 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, therefore it is necessary to implement human health protection measures in accordance with good laboratory practice guidelines. It is mandatory to read and act according to the information and warning signs printed on the product label, instructions for use and in the safety data sheet, which is available on request.

**Storage, transport, stability, and shelf life**

Upon receipt, store the product in a dry, well-closed original packaging at a temperature of +15 °C to +25 °C. After opening the BioSchiff reagent for the first time, keep BioSchiff reagent at a temperature of +2 °C to +8 °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 production date and expiration date are printed on the product label.

**Literature**

1. Culling, CFA(1974): Handbook of histopathological and histochemical techniques, 2nd ed., Butterworth, London, UK.
2. Davey, FR et Nelson, DA(1977): Periodic Acid Schiff (PAS) Stain. IN Hematology, 2<sup>nd</sup> ed., WJ Williams, E. Buettler, AJ Erslev, RW Rundles, McGraw-Hill, New York, p. 1630-1632.
3. Hotchkiss, RD(1948): A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations, *Arch. Biochem.* 16, p. 131.
4. Sheehan DC et Hrapchak, BB(1980): Theory and Practice Histotechnology, 2<sup>nd</sup> ed., CV Mosby, St. Louis, (MO), pp 52, p. 14-167.

Warnings and precautions regarding the materials contained in the product:	
	<p>H225 H319 H336 EUH031</p> <p>P21 P280 P305+P351+P338</p> <p>P308+P313 P305+P351+P338</p> <p>Highly flammable liquid and vapour.                      Causes severe eye irritation.                      May cause drowsiness or dizziness.                      Releases toxic gas in contact with acids.</p> <p>Keep away from heat, hot surfaces, sparks, open flames and other sources of ignition. Do not smoke.                      Wear protective gloves/protective clothing/eye protection/face protection.                      IF IN EYES: rinse cautiously with water for several minutes. Remove contact lenses if you wear them and if they are easily removed. Continue rinsing.</p> <p>IF EXPOSED OR SUSPECTED OF EXPOSURE: Get medical advice/attention.                      IF IN EYES: rinse cautiously with water for several minutes. Remove contact lenses if you wear them and if they are easily removed. Continue rinsing.</p>

AB-IFU, EN12, 06.06.2024., LO/ISP

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

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Version	Description / reason for change	Date
12.	Revised in accordance with Regulation (EU) 2017/746 - IVDR	06.06.2024.