ALCIAN BLUE-P.A.S. KIT

IVD *In vitro* diagnostic medical device CE

Seven-reagent kit for determining acid mucopolysaccharides according to Mowry

INSTRUCTIONS FOR USE

REF Product code: AB-100T (for 100 tests) AB-K-100 (7 x 100 mL) AB-K-500 (7 x 500 mL)

Introduction

One of the most frequently used chemical methods in histology is P.A.S. staining. Combined with Alcian Blue pH 2.5 solution on a single section enables differentiation between neutral and acid mucins, glycogens and glycoproteins. Alcian Blue dye stains acid mucins that turn insoluble and resistant to remaining reagents during P.A.S. staining procedure. Oxidizing properties of periodic acid enable characteristic magenta staining combined with BioSchiff reagent. Nuclei are stained with Hematoxylin ML (Mayer-Lillie) that does not interfere with Alcian Blue dye.

Product description

- **ALCIAN BLUE-P.A.S. KIT** – Kit for staining neutral and acid mucins, glycogens and glycoproteins.

The kit contains:	100 tests (AB-100T)	7 x 100 mL (AB-K-100)	7 x 500 mL (AB-K-500)
Alcian Blue solution pH 2.5	30 mL (AB2-OT-30)	100 mL (AB2-OT-100)	500 mL (AB2-OT-500)
Sodium tetraborate, solution	30 mL (NTB-OT-30)	100 mL (NTB-OT-100)	500 mL (NTB-OT-500)
Periodic acid, 0.8% solution	30 mL (PK08-OT-30)	100 mL (PK08-OT-100)	500 mL (PK08-OT-500)
BioSchiff reagent	30 mL (BS-OT-30)	100 mL (BS-OT-100)	500 mL (BS-OT-500)
Sodium metabisulphite, solution	30 mL (NM-OT-30)	100 mL (NM-OT-100)	500 mL (NM-OT-500)
HCL reagent, P.A.S.	30 mL (HCLP-OT-30)	100 mL (HCLP-OT-100)	500 mL (HCLP-OT-500)
Hematoxylin ML	30 mL (HEMML-OT-30)	100 mL (HEMML-OT-100)	500 mL (HEMML-OT-500)

Preparation of additional solutions used in staining

- Sulfite solution
  Mix 10 ml of Sodium metabisulfite, solution with 10 ml of HCL reagent, P.A.S. Add another 200 ml of tap water, then mix.
  Note: Prepare the sulfite solution shortly before using.

Preparing histological sections for staining

- Fixate the sample (Formaldehyde NB 4%, Formaldehyde NB 10%), 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 μm 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 (AB-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 ≥5 drops)	30 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 under tap water	5 min
8.	Rinse in distilled (demi) water	1-2 minutes
9.	Treat with Periodic acid, 0.8% solution (≥5 drops)	5-10 minutes
10.	Rinse under tap water	3 min
11.	Rinse the section with distilled (demi) water	
12.	Treat with BioSchiff reagent (add ≥5 drops)	10-15 minutes
13.	Treat with sulfite solution (add ≥5 drops)	3 exchanges, 2 min each
14.	Rinse under tap water	3 min
15.	Stain using Hematoxylin ML (add ≥5 drops)	1-3 min
16.	Rinse under tap water	3 min
17.	Dehydration using 70% alcohol (Histanol 70)	5 dips
18.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
19.	Dehydrate using 100% alcohol (Histanol 100)	2 min
20.	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 seven-reagent 100 mL or 500 ml kit (AB-K-100, AB-K-500)

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	30 min
6.	Without rinsing, immerse into Sodium teraborate, solution	10 min
7.	Rinse under tap water	5 min
8.	Rinse in distilled (demi) water	1-2 minutes
9.	Immerse into Periodic acid, 0.8% solution.	5-10 minutes
10.	Rinse under tap water	3 min
11.	Rinse the section with distilled (demi) water	
12.	Immerse in BioSchiff reagent	10-15 minutes
	Note: during staining procedure it is required to put a lid on the jar in order to avoid SO_2 evaporation	
13.	Without rinsing, immerse into sulfite solution	3 exchanges, 2 min each
14.	Rinse under tap water	3 min
15.	Immerse into Hematoxylin ML	1-3 min
16.	Rinse under tap water	3 min
17.	Dehydration using 70% alcohol (Histanol 70)	5 dips
18.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
19.	Dehydrate using 100% alcohol (Histanol 100)	2 min
20.	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

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

Note

Time periods of staining processes are not entirely standardized and they approximately correspond to clinical and laboratory practical experience. Intensity of staining depends on the period of immersion in the dye. Real staining protocol depends on personal requests and priorities.

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 Alcian Blue-P.A.S. kit in a tightly sealed original packaging at temperature of +15 to +25°C. Do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's label. Valid BioSchiff reagent solution is colorless. Discard after it starts to assume color because of the SO_2 loss.

References

1. Culling, C.F.A.(1974): Handbook of histopathological and histochemical techniques, 2nd ed., Butterworth, London, UK.
2. Davey, F.R. et Nelson, D.A.(1977): Periodic Acid Schiff (PAS) Stain. IN Hematology, 2nd ed., W. J. Williams, E. Buetler, A. J. Erslev, R.W. Rundles, McGraw-Hill, New York, p 1630-1632.
3. Hotchkiss, R.D.(1948): A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations, *Arch. Biochem.* 16, p 131.
4. Sheehan D.C. et Hrapchak, B.B.(1980): Theory an Practice Histotechnology, 2nd ed., CV Mosby, St. Louis, (MO), pp 52, p 14-167.

AB-X, V10-EN9, 02 December 2019, IŠP/VR

	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	CE
	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						