

ALCIAN BLUE SOLUTION pH 2.5

IVD *In vitro* diagnostic medical device

CE

Solution for staining acid mucopolysaccharides

INSTRUCTIONS FOR USE

REF Product code: AB2-OT-100 (100 mL)

AB2-OT-500 (500 mL)

AB2-OT-1L (1000 mL)

Introduction

Alcian Blue 8GX 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 solution pH 2.5 is a component of Alcian Blue-P.A.S. kit, as well as Alcian Blue pH 2.5 kit. Alcian Blue pH 2.5 kit enables adequate staining and visualization of acid mucins without staining sulfated mucins. Alcian Blue-P.A.S. kit enables differentiation between neutral and acid mucins, glycogens and glycoproteins.

Product description

- **ALCIAN BLUE SOLUTION pH 2.5** – Alcian Blue 8GX solution of optimal concentration and pH set at 2.5

Example of staining using Alcian Blue solution pH 2.5 as a component of Alcian Blue - P.A.S. kit:

| Alcian Blue-P.A.S. 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 μ m slices and place them on a VitroGnost glass slide

NOTE

Apply the reagent so it completely covers the section.

Procedure for staining the section using the 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 \geq 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 \geq 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 (add \geq 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 \geq 5 drops) | 10-15 minutes |
| 13. | Treat with sulfite solution (add \geq 5 drops) | 3 exchanges, 2 min each |
| 14. | Rinse under tap water | 3 min |
| 15. | Stain using Hematoxylin ML (add \geq 5 drops) | 1-3 min. |
| 16. | Rinse under tap water | 3 min |
| 17. | Dehydrate 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 – teal blue

PAS 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 solution pH 2.5 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.

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. Buettler, 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.

AB2-X, V1-EN1, 8 January 2016, IŠP/VR

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|  | 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

