

Fixation and fixatives

[Science](#), [Biology](#)



Alcian Blue Rationale for Use

Alcian blue is a water-soluble, amphoteric copper phthalocyanine, which is most often used as a basic dye. In general, alcian blue has an affinity for sulfated and carboxylated groups of acid mucopolysaccharides, but the specific group demonstrated is dependent on the dye solution pH.

Recommended Fixatives

10% neutral buffered formalin and Bouin's fixative.

Avoid

Chromate Fixatives

Mode of Action (1, 2, 3)

Alcian blue binds electrostatically with the acidic groups in the mucopolysaccharide molecule.

The components to be demonstrated are intensely stained if the dye solution is used at the specific pH at which the reactive groups are fully ionized. To achieve full ionization of the reactive groups, some alcian blue methods begin with a rinsing of sections in the appropriate pH solution prior to staining with the dye solution. In general, strongly sulfated mucins react more consistently at low pH levels. Above a pH of 1.0, their reactions are variable. At a pH of 0.2 only strongly sulfated mucosubstances will be demonstrated, while weak sulfated mucins stain well between a pH of 1.0 - 2.5, and often below 1.0. Carboxylated mucins react with alcian blue at pH 2.5. Alcian blue produces an intense greenish-blue or teal coloration. To ensure this color is not lost during subsequent chemical and dye treatments,

such as those found in the Alcian Blue-Verhoeff's van Gieson procedure and Movat's Pentachrome, alcian blue is converted to an insoluble pigment. By exposing alcian blue-stained sections to a pre-heated 80°C alkaline alcohol solution, alcian blue is converted to the insoluble pigment, Monastrell fast blue. Further exposure of the sections to various decolorizers and dyes, fails to change the alcian blue intensity.

Quality Control and Control Materials

1. Appropriate control tissues include the colon and small intestine. If lung sections contain bronchi with mucous glands, are used as a control, the tech should be aware that the cartilage matrix will also stain at approximately pH 2.5.
2. To avoid the coloration of hyaluronic acid found in connective tissue matrix or amorphous ground substance, the tissue section can first be treated with a solution of testicular streptococcal hyaluronidase before staining with alcian blue. Testicular hyaluronidase requires only 2 hours of incubation versus 24 hours with streptococcal hyaluronidase.
3. Alcian blue powder solubility may decrease after storage of more than three years, but good staining results have been reported with dye lots older than three years (4).
4. The dye solution pH is critical to ensure the demonstration of specific chemical groups. pH 0.2 -only strongly sulfated mucins demonstrated
pH 1.0 -strongly and weakly sulfated mucins
pH 2.5 -carboxylated and weakly sulfated mucins
5. To ensure the quality of staining at the appropriate pH, the section may be rinsed in the solvent solution prior to staining in the dye, i. e. ,

rinsing in pH 2.5, 3% aqueous acetic acid solution prior to placing the sections in the pH 2.5 alcian blue solution, which is prepared by combining alcian blue dye powder in a 3% aqueous acetic acid solution.

6. Some procedures indicate it is important to avoid rinsing the stained slides in water after treatment in alcian blue dye. Blotting the slides dry after staining is recommended.
7. It is important to avoid celloidinization of tissue sections because alcian blue has a strong affinity for celloidin.
8. A white haze may appear on the back of the glass slide after staining with nuclear fast red and contact with the air. Moistening a kimwipe or facial tissue with saliva and rubbing the back of the glass will remove the haze. Follow this with the cleaning of the glass using a kimwipe moistened with 100% ethanol to remove the saliva.
9. Cut paraffin sections at 4-6 microns.

References

1. Bancroft J. D. and Stevens A.: Theory and Practice of Histological Techniques, 2nd edition. Churchill Livingstone, 1982.
2. Kiernan J. A.: Histological and Histochemical Methods: Theory and Practice, Pergamon Press, 1981.
3. Sheehan D. C. and Hrapchak B. B.: Theory and Practice of Histotechnology, 2nd edition, C. V. Mosby Co., 1980.
4. Shrenk E.: Note from the Biological Stain Commission—a newly certified dye-Alcian blue 8GX. Stain. Tech. 56(3), 129, 1981.

Alcian Blue

pH 2.5

Solutions

- a. 1% Alcian Blue Solution - pH 2.5 Alcian Blue 8GX (C. I. 74240) 1gm 3% Glacial acetic acid 100ml (3 ml. glacial acetic acid to 97 ml. distilled water)
Add a crystal of thymol to prevent mold growth. The solution can be filtered and reused.
- b. Nuclear Fast Red (Kernechtrot) Solution (pg. 19)

Procedures (1, 2)

1. Deparaffinize and hydrate slide to distilled water. (See Note 1)
2. Stain in alcian blue solution for 30 minutes. Filter solution back for reuse. (See Note 2)
3. Wash for 2 minutes in running tap water.
4. Rinse in distilled water.
5. Counterstain in nuclear fast red for 3 to 5 minutes. Quickly transfer slides to distilled water. Filter solution back for reuse.
6. Rinse slides in distilled water.
7. Dehydrate, clear, and mount. If a cloudy haze appears on the side refer to quality control information, #8.

Color Results

Carboxylated and weakly sulfated acid mucins - light blue to medium greenish-blue Nuclei-reddish pink.

Note

1. After step 1, the slides may be placed in a pH 2.5, 3% aqueous acetic solution for 1 minute, then proceed to step 2.
2. After step 2, the slides can be blotted dry, instead of rinsing in running tap water and distilled water. Continue the procedure with step 5.

References

1. Lev R. and Spicer S. S.: Specific staining of sulfate groups, with Alcian blue at low pH. *J. Histochem. Cytochem.* , 12: 309, 1964.
2. Sheehan D. C. and Hrapchak B. B.: *Theory and Practice of Histotechnology*, 2nd edition. C. V. Mosby Co., 1980