

## Alcian Blue (pH 2.5) Stain Kit

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### Intended Use

For In Vitro Diagnostic Use

### Summary and Explanation

The Alcian Blue (pH 2.5) Stain Kit is intended for use in the histological visualization of sulfated and carboxylated acid mucopolysaccharides and sulfated and carboxylated sialomucins (glycoproteins).

Acidic Sulfated Mucosubstances: Blue

Hyaluronic Acid: Blue

Sialomucins: Blue

Nuclei: Red

Background: Pink

### Control Tissue

Tissues fixed in 10% formalin are suitable for use prior to paraffin embedding. Consult references (Kiernan, 1981; Sheehan & Hrapchak, 1980) for further details on specimen preparation.

1. Cut sections, usually 3 to 5  $\mu\text{m}$  and pick the sections up on glass slides.
2. Bake the slides for at least 30 minutes at approximately 70°C.
3. Allow to cool.

### Recommended Positive Control

1. Small Intestine
2. Appendix
3. Colon

### Reagents Provided

Kit Contents	Volume	Storage
Alcian Blue, pH 2.5	250 mL	15-30°C
Safranin O Solution	250 mL	15-30°C
3% Acetic Acid Solution	500 mL	15-30°C

### Storage and Handling

Do not use product after the expiration date printed on vial. If reagents are stored under conditions other than those specified here, they must be verified by the user. Diluted reagents should be used promptly.

### Staining Procedure

1. Deparaffinize sections if necessary and hydrate to distilled water.
2. Incubate slide in Acetic Acid solution for 3 minutes.
3. Incubate slide in Alcian Blue (pH 2.5) solution for 30 minutes at room temperature or 15 minutes at 37°C.
4. If desired, rinse slide briefly in Acetic Acid solution to remove excess Alcian Blue.
5. Rinse for 2 minutes in running tap water followed by 2 changes of distilled water.
6. Stain slide in Safranin O Solution for 5 minutes.

7. Rinse for 2 minutes in running tap water followed by 2 changes of distilled water.
8. Dehydrate through graded alcohols.
9. Clear, and mount in synthetic resin.

### Limitations of the Procedure

1. Histological staining is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selections, fixation, processing, preparation of the slide, and interpretation of the staining results.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining.
3. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts or false negative results.
4. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology and other histopathological criteria. It is the responsibility of a qualified pathologist to be familiar with the special stain and methods used to produce the slide.
5. Staining must be performed in a certified licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.

### Precautions

1. Consult local and/or state authorities with regard to recommended method of disposal.
2. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
3. Avoid microbial contamination of reagents. Contamination could produce erroneous results.
4. This reagent may cause irritation. Avoid contact with eyes and mucous membranes.
5. If reagent contacts these areas, rinse with copious amounts of water.
6. Do not ingest or inhale any reagents.
7. Use in a chemical fume hood whenever possible.

### Troubleshooting

If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem is suspected, contact Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or [techsupport@dbiosys.com](mailto:techsupport@dbiosys.com).

### References

- I. Lillie, R.D. 1977, H.J. Conn's Biological Stains, 9th Edition. Williams & Wilkins, Baltimore. Pages 452-455.
- II. Sheenan, D.C., Hrapchak, B.B. Theory and Practice of Histotechnology, 2nd Edition. Battelle Press, Columbus, OH. Pages 172-173.
- III. Churukian, C.J., 1989, Manual of Special Stains Laboratory, 4th Edition. University of Rochester, Rochester, New York. Pages 55-56.
- IV. Carson, F.L., 1996, Histotechnology; A Self-Instructional Text, 2nd Edition. ASCP Press, Chicago, IL. Pages 117-121.

