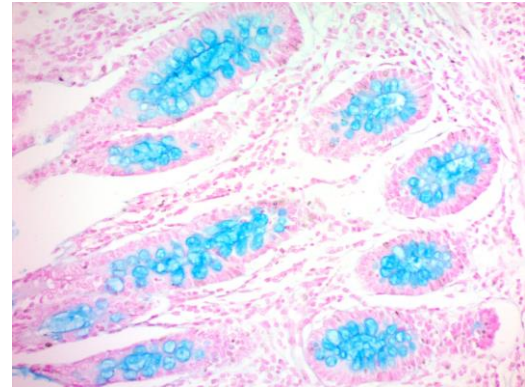


# Alcian Blue (pH 1.0) Stain Kit

**Description:** The Alcian Blue (pH 1.0) Stain Kit is intended for use in the histological visualization of strongly sulfated mucosubstances. Alcian blue staining at pH 1.0 differs from staining at pH 2.5, in that carboxylated mucins are protonated and only sulfated mucins are still ionized and therefore stained.

Strongly Sulfated Mucosubstances: Blue  
Nuclei: Red  
Background: Pink



**Uses/Limitations:** Not to be taken internally.  
For In-Vitro Diagnostic use only.  
Histological applications.  
Do not use past expiration date.  
Use caution when handling reagents.  
Non-Sterile.

**Control Tissue:** Tissue known to be positive for sulfated mucins. e.g. deep mucosa of colon

**Availability/Contents:**

<u>Item #</u>	<u>Kit Contents</u>	<u>Volume</u>	<u>Storage</u>
ANA250	Alcian Blue Solution (pH 1.0)	250 ml	18-25°C
HQA500	Hydrochloric Acid Solution (1N)	500 ml	18-25°C
NFS250	Nuclear Fast Red (Enhanced Stability)	250 ml	18-25°C

**Precautions:** Avoid contact with skin and eyes.  
Harmful if swallowed.  
Follow all Federal, State, and local regulations regarding disposal.  
Use in chemical fume hood whenever possible.


**Procedure notes:** Maintaining proper pH is critical to preventing false-positive mucin staining. 'Working Rinse Solution' should fall within pH 1.0 ± 0.15 and is used both before and after the Alcian Blue to control pH. Rinsing with deionized water or any other rinse may affect pH and cause non-specific staining. We recommend running at least one slide without the counterstain to compare intensity with slides that have been counterstained. Below procedure is written for slides laying horizontally that are stained by applying a small amount of solution.

**Procedure**

1. Deparaffinize sections if necessary and hydrate to distilled water.
2. Make up sufficient amount of 'Working Rinse Solution' by mixing the following:
  - 1 part** of Hydrochloric Acid Solution (1N)
  - 9 parts** Deionized water

*Notes: -An example mixture would be 10mls Hydrochloric Acid Solution + 90mls Deionized Water*  
*-We suggest making at least 10mls per slide. A smaller amount is required for step 3 and a larger amount used in step 5 for rinsing.*

Storage: 18° C  25° C

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3. Apply a small amount (<2ml/slide) of 'Working Rinse Solution' to tissue for 30 seconds to adjust pH in preparation for staining. Save remaining 'Working Rinse Solution' for step 5.
4. Drain slide and without rinsing, stain tissue section with Alcian Blue Solution (pH 1.0) solution for 30 minutes
5. Quickly and thoroughly rinse excess stain off slide using remaining 'Working Rinse Solution'.
6. Carefully blot and allow slide to air dry.
7. If preferred, counter stain in Nuclear Fast Red (Enhanced Stability) for 1-2 minutes with occasional agitation. Rinse very briefly in deionized water and allow to completely air dry again.
8. Clear, and mount in synthetic resin.

**References:**

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

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