Diagnostic BioSystems

Instructions For Use

KT 002-IFU

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Alcian Blue (pH 1.0) Stain Kit

Description and Principle

The Alcian Blue (pH 1.0) Stain Kit is intended for use in the histological visualization of strongly sulfated mucosubstances.
Alcian Blue, a copper phthalocyanine dye, binds acid mucosubstances. When used in a pH 1.0 acid solution Alcian Blue selectively stains sulfated acid mucosubstances. Acid mucins that are carboxylated only are protonated and will not be stained.

Expected Results

Strongly Sulfated Mucosubstances:	Blue
Nuclei:	Red
Background:	Pink

Kit Contents

	Volume	Storage
1. Alcian Blue Solution (pH 1.0)	250 ml	15-30°C
2. Hydrochloric Acid Solution (1N)	500 ml	15-30°C
3. Nuclear Fast Red (Enhanced Stability)	250 ml	15-30°C

Suggested Controls *(not provided)*

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

Uses/Limitations

For In-Vitro Diagnostic use only.
Do not use if reagents become cloudy or precipitate
Do not use past expiration date.
Use caution when handling reagents.
Non-Sterile
Intended for FFPE sections cut at 5-10µm.
This procedure has not been optimized for frozen sections.
Frozen sections may require protocol modification.

Storage

Store kit and all components at room temperature (15-30°C).

Safety and Precautions

Please see current Safety Data Sheets (SDS) for this product and components GHS classification, pictograms, and full hazard/precautionary statements. If there is any serious incident that has occurred in relation to the device, please contact the manufacturer: Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or techsupport@dbiosys.com. If required, please report to the Competent Authority of the Member State in which the user and/or patient is established.

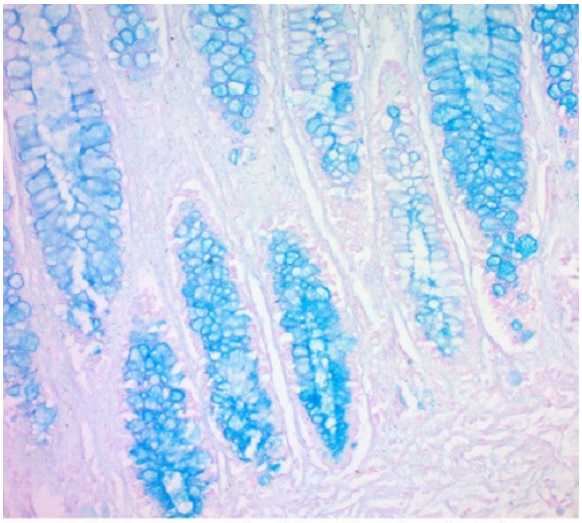
Procedure notes:

1. 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.

2. In tissues with low-to-moderately sulfated mucins, such as human stomach, the addition of Methanol and Sodium Chloride (neither provided) may be need to be added to the post rinsing solution (step 5) to prevent nonspecific staining for accurate relative quantification[6]. First rinse in 0.1N HCl (Working Rinse Solution) containing 10% (v/v) Methanol three times then rinse three times with 0.1N HCl containing 0.5M NaCl.

Procedure

1. Deparaffinize sections if necessary and hydrate to distilled water.



Sulfated Mucins stained with Alcian Blue Solution (pH 1.0) on Mouse Intestine. Magnification 200X

2. Make up sufficient amount of 'Working Rinse Solution' (0.1N HCl) 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.

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.

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References

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2. Churukian, C.J., 1989, Manual of Special Stains Laboratory, 4th Edition. University of Rochester, Rochester, New York. Pages 55-56.
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