## ALCIAN BLUE pH 2.5 METHOD FOR MUCOSUBSTANCES (Microwave)

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of acid mucopolysaccharides.
PRINCIPLE:	Alcian Blue at pH 2.5 binds to the acidic groups of sulfated and carboxylated glycoproteins and mucin by means of salt bridges. Acetic Acid provides the acidic environment necessary to facilitate these bonds.
CONTROL:	Small intestine, appendix or colon Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs018.
SPECIMEN PREPARATION:	Formalin fixed, paraffin embedded sections cut at 6 micrometers
SOLUTIONS:	<ol> <li>Acetic Acid 3% Aqueous Item# s101</li> <li>Alcian Blue 1% in 3% Acetic Acid pH 2.5 Item# s111A</li> <li>Nuclear Fast Red Kernechtrot 0.1% Item# s248</li> <li>Solutions can be purchased separately from Poly Scientific.</li> </ol>
NOTES:	Variations in timing may occur due to the power wattage of the microwave oven. Provided times and power levels are based on 1000 watt microwave oven.
REFERENCE:	Luna, Lee G. <u>Histopathologic Methods and Color Atlas of Special</u> Stains and Tissue Artifacts. American Histolabs Inc. Gaithersburg, MD. 1992. pp. 377, 577.

## STAINING PROCEDURE:

- 1. Deparaffinize and hydrate to distilled water.
- 2. Place 50 mL of Acetic Acid 3% Aqueous in plastic coplin jar (loosely apply cap) and microwave for 20 seconds. Stir and re-microwave for an additional 15 seconds or until solution is hot. Stir again and place slides in hot solution for 1 minute.
- 3. Place 50 mL of Alcian Blue 1% in 3% Acetic Acid pH 2.5 in plastic coplin jar (loosely apply cap) and microwave for 20 seconds. Stir and re-microwave for 15–20 seconds or until solution is hot. Stir again and add slides to hot solution for 2 minutes. Check control. If additional staining time is needed place slides back in hot solution until desired intensity is reached.
- 4. Rinse well in distilled water.
- Place 50 mL of Nuclear Fast Red Kernechtrot 0.1% in plastic coplin jar for 15 seconds. Stir again and add slides to hot solution for 1 1/2-2 minutes or until desired intensity is reached.
- 6. Rinse well in distilled water.
- 7. Dehydrate, clear and mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

## **RESULTS**:

Weakly Acidic Sulfated Mucosubstance, Hyaluronic Acid and Sialomucins... Dark Blue

Background ...... Red

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