ALCIAN BLUE pH2.5 - ACID MUCOPOLYSACCHARIDES

PURPOSE: Alcian blue stains acid mucosubstances and acetic mucins. Excessive amounts of non-sulfated acidic mucosubstances are seen in mesotheliomas, certain amounts occur normally in blood vessel walls but increase in early lesions of atherosclerosis.

PRINCIPLE: Alcian blue is a group of polyvalent basic dyes that are water soluble. The blue color is due to the presence of copper in the molecule. The 3% acetic acid solution (pH2.5), Alcian blue stains both sulfated and carboxylated acid mucopolysaccharides and sulfated and carboxylated sialomucins (glycoproteins). It is believed to form salt linkages with the acid groups of acid mucopolysaccharides.

CONTROL: Small intestine, appendix, or colon.

FIXATIVE: 10% NBF, Bouin's, or Hollande's.

TECHNIQUE: 4m paraffin sections.

EQUIPMENT: Rinse glassware in DI water. Coplin jars, pH meter, microwave.

REAGENTS:

3% Glacial Acetic Acid

Acetic acid	3.0 ml
Distilled water	100.0 ml

Solution is stable for 1 year.

CAUTION: Contains acid.

Alcian Blue Solution:

3% glacial acetic acid	100.0 ml
Alcian blue 8GX	1.0 gm

Mix, adjust pH to 2.5, using acetic acid. Filter, add a crystal of thymol, label with initial and date. Solution is stable for 2 to 6 months.

CAUTION: Contains acid, avoid contact and inhalation of dye.

Nuclear Fast Red (Kernechtrot):

Aluminum sulfate	25.0 gm
Distilled water	500.0 ml
Nuclear fast red	0.5 gm

Dissolve the aluminum sulfate in the water. Add the nuclear fast red, dissolve with aid of heat. Filter, add a crystal of thymol. Stable for 1 year.

CAUTION: IRRITANT avoid contact and inhalation

SAFETY: Wear gloves, goggles and lab coat. Add acid to water. Call Safety Services for spills over 500ml, contain spills with ACID SPILL KITS. Prepare stain in fume hood. Avoid contact and inhalation of dyes.
Acetic acid: Irritating to respiratory system. Target organ effects on respiratory system by inhalation. Corrosive.

PROCEDURE:

1. Hydrate slides to distilled water.
2. 3% acetic acid, 3 minutes.
3. *Alcian blue solution, microwave: Hi power, 30 seconds.
4. Wash in running water for 2 minutes, rinse in distilled.
5. Nuclear-fast red, 5 minutes, wash in tap water.
6. Dehydrate, clear, and coverslip.

*Conventional Method: Alcian blue, room temperature for 30 minutes.

RESULTS:

Acid mucins/mucosubstances:	blue
Nuclei (using Nuclear fast red)	reddish pink

REFERENCES:

Sheehan D, Hrapchak B, Theory and practice of Hlstotechnology, 2nd Ed, 1980, pp 163,173-174, Battelle Press, Ohio

Bancroft J, Stevens A, Theory and Practice of Histological Techniques, 2nd Ed, 1982,pp194-98,Churchill Livingstone, N.Y.

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Crookham,J, Dapson,R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech

Prepared:_____By: _____

Approved:_____By:_____

PROCEDURE CARD

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DATE:_____

TECH:_____

EXPIRATION:_____

ALCIAN BLUE pH2.5

DATE:_____

TECH:_____

3% GLACIAL ACETIC ACID

Acetic acid	3.0 ml
Distilled water	100.0 ml

Solution is stable for 1 year.

CAUTION: Acid, avoid contact or inhalation.

DATE:_____

TECH:_____

EXPIRATION:_____

3% ACETIC ACID

DATE:_____

TECH:_____

NUCLEAR FAST RED (Kernechtrot):

Aluminum sulfate	25.0 gm
Distilled water	500.0 ml
Nuclear fast red	0.5 gm

Dissolve the aluminum sulfate in the water. Add the nuclear fast red, dissolve with aid of heat. Filter, add a crystal of thymol. Stable for 1 year.

CAUTION: Irritant, avoid contact and inhalation.

TECH:_____

DATE:_____

EXPIRATION:_____

NUCLEAR-FAST RED

DATE:_____

TECH:_____