

Alcian Blue and Nuclear Fast Red Staining on Sections

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Description: 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 increased in early lesions of atherosclerosis. Strongly acidic mucosubstances will be stained blue, nuclei will be stained pink to red, and cytoplasm will be stained pale pink.

Fixation: formalin or PFA fixed, paraffin embedded tissue sections.

Solutions and Reagents:

3% Acetic Acid solution:

Glacial Acetic Acid	7.5 ml
Distilled water	242.5 ml

Alcian Blue solution (pH 2.5)

Alcian Blue, 8GX (Sigma, A5268)	2.5 g
Acetic Acid solution 3%	250 ml

Mix well and adjust pH to 2.5 using acetic acid.

0.1% Nuclear Fast Red, 5% aluminum sulfate solution:

Nuclear Fast Red (Sigma, N8002)	0.25 g
Aluminum sulfate (Sigma, A7523)	12.5 g
Distilled water	250 ml

Dissolve aluminum sulfate in water. Add nuclear fast red and slowly heat to boil and cool. Filter and add a grain of thymol (Sigma, T0501) as a preservative.

Procedure:

1. Deparaffinize slides by clearing in xylene or xylene substitute, 2x 3 min
2. Hydrate to distilled water: 100% EtOH, 2x 2 min
3. Hydrate to distilled water: 90% EtOH, 70% EtOH, and dH₂O, 1x 2 min each.
4. Incubate slides in 3% acetic acid for 3 min.
5. Stain in Alcian Blue solution for 30-45 min.
6. Wash in running tap water for 2 min.
7. Counter stain in nuclear fast red solution for 3-5 min.
8. Wash in running tap water for 1 min.
9. Rinse in distilled water, 1x 2 min.
10. Dehydrate slides: 70%, 95%, and 100% EtOH, 1x 2 min each.
11. Clear in xylene or xylene substitute, 2x 3 min.
12. Mount with resinous mounting medium (PermOUNT, Fisher Scientific SP15-500).

Results:

Strongly acidic sulfated mucosubstances	Blue
Nuclei	Pink to red
Cytoplasm	Pale pink