

Protocol for Alcian Blue Staining for Frozen Sections

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Alcian Blue pH 1.0 staining for strongly sulfated mucosubstances (chondroitin, dermatan, keratan sulphate) (**Usually use this one**)

Alcian Blue GX 1g
0.1M HCl 100ml

Alcian Blue pH 2.5 for Hyaluronic acid, acid mucins, sulfated mucosubstances

Alcian Blue 1g
3% Acetic Acid 100ml (glacial acetic acid 3ml, H₂O 97ml)

Method:

- 1) Fix slides with 0.1% glutaraldehyde or Kahle's for 4 hours at room temperature (**Note: fix 20min for cultured cells**).
- 2) rinse 3 times with 1x PBS
- 3) stain in alcian blue solution for overnight (can try 30 min)
- 4) rinse with 0.1M HCl (solvent of stain) x1
- 5) rinse twice with 1x PBS
- 6) rinse with water
- 7) mount (use Biomedex Gel/Mount, Cat# M01, Fisher, no Permount directly on OPLA as toluene will dissolve it)
- 8) seal edges of coverslip with permount

Note: For OPLA (from BD Discovery Lab)

Fix with Glutaraldehyde 1%, stain with Alcian blue overnight before sectioning

Strongly sulfated mucosubstances:	Blue
Nuclei:	pink to red
Cytoplasm and background:	pale pink