

**Alcian Blue Staining for Micromass Cell Culture**  
**James Bullock 08/04/05**

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, H<sub>2</sub>O 97ml)

**Method:**

- 1) Fix cells with 0.1% glutaraldehyde in PBS (or lab's fixative solution for Alkaline phosphatase staining) for 20min at room temperature.
- 2) rinse 3 times with 1x PBS
- 3) stain in alcian blue solution for 1-2hrs or overnight.
- 4) rinse with 0.1M HCl (solvent of stain) x1
- 5) rinse twice with 1x PBS
- 6) keep in ddH<sub>2</sub>O or PBS

**Staining results:**

Strongly sulfated mucosubstances:      Blue

Nucei: pink to red

Cytoplasm and background:      pale pink