

Alcian blue staining of limb bud micromass cultures

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Previously described in H.-S. Seo. R. Serra, Dev Biol, 2007, 310(2):304-316

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Reagents/solutions:

- 1) 0.05% Alcian blue staining solution in 75% EtOH: 0.1M HCl (4:1)
 - a. Alcian blue 8 GX, Sigma, Cat# A5268
- 2) Fixative solution (30% EtOH, 0.4% paraformaldehyde (PFA), 4% acetic acid):
 - a. To make 50 ml, add:
 - i. 15 ml of 100% EtOH
 - ii. 2 ml of glacial acetic acid
 - iii. 5 ml of 4% PFA
- 3) 4% PFA
 - a. Dissolve 2 g of paraformaldehyde in 50 ml of warm PBS, let cool to room temperature
- 4) 6 M guanidine hydrochloride
 - a. Guanidine hydrochloride, Sigma, Cat# G3272, MW 95.53
 - b. Dissolve 57.318 g of guanidine hydrochloride in 100 ml of ddH₂O

Procedure:

- 1) Aspirate media from micromass cultures
- 2) Fix with 500 μ l of Fixative solution for 15 min at room temperature
- 3) Stain with alcian blue staining solution overnight at 37 °C (~500 μ l/well of a 24-well dish)
- 4) Wash off dye with double distilled H₂O
 - a. Can extract the dye by incubating cultures with 250 μ l of 6M guanidine hydrochloride overnight at room temperature
 - b. Measure the optical density at OD₅₉₅