

# Alcian Blue Cartilage Staining

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1. Embryos were dissected from the uterus in 1X phosphate buffer solution (PBS) and fixed individually in Bouin's Solution for 2 hours at room temperature. It is not necessary to remove any viscera in embryos at early stages, however, older embryos will require evisceration due to the presence of blood and dark organs.
2. The extraembryonic membranes of each embryo were labeled, frozen and saved for genotyping of the embryos, if necessary.
3. The embryos were rinsed in 70% ethanol + 0.1% NH<sub>4</sub>OH in 15 ml polypropylene centrifuge tubes for 6 to 8 washes over a 24 hour period until embryos appear white.
4. Change the embryos into 5% acetic acid washes twice for 1 hour each.
5. Stain embryos for 2 hours (larger embryos will require more time) in 0.05% Alcian blue 8GX (Fisher) in 5% acetic acid.
6. Wash embryos in 5% acetic acid for 2X 1 hour each.
7. Clear embryos in 100% methanol 2X for 1 hour each.
8. Transfer embryos to polyethylene scintillation vials and incubate in 2:1 benzyl benzoate: benzyl alcohol (3 hour plus overnight). (Be careful using benzyl benzoate: benzyl alcohol because it dissolved plastics.)

## Solutions:

### 1X PBS

8 g NaCl  
.2 g KCl  
1.44 g Na<sub>2</sub>HPO<sub>4</sub>  
.24 g KH<sub>2</sub>PO<sub>4</sub>  
800 ml DDW

Dissolve chemicals, pH to 7.4, add DDW to 1 L, DEPC treat and autoclave.

### Alcian Blue

0.05% Alcian blue 8GX  
5% acetic acid  
water

### Bouin's Fixative

75 ml saturated picric acid  
25 ml 40% formaldehyde  
5 ml glacial acetic acid  
Cover in foil and store at room temperature.

