

Cartilage Staining of Mouse Embryos

(Rivera lab)

(Adapted from Jegalian and De Robertis, 1992. *Cell* 71, 901)

Used with good results in embryos between 13.5 – 16.5 days.

Dissect embryos in PBS and remove extra-embryonic membranes (use them for genotyping if necessary).

1. Fix in Bouin's solution for two hours.
2. Rinse with a solution of 70% ethanol plus 0.1% NH₄OH over a course of 24 hours until embryos appear white. Six to eight changes may be required.
3. Equilibrate with 5% acetic acid. Two changes, each for one hour.
4. Stain for two hours with 0.05% Alcian Blue 8GX (Fisher) in 5% acetic acid.
5. Rinse twice with 5% acetic acid for one hour each.
6. Dehydrate embryos by incubating twice in methanol one hour each.
7. Clear in 1:2 benzylalcohol:benzyl benzoate (BABB). Use glass containers when using BABB.

* In older embryos (15.5-16.5) evisceration may be required.

** Staining has been shown to last at least two years in BABB. It is advisable to document the results immediately if they are relevant.