

Alcian Blue – Alizarin Red Skeletal Staining

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1. Dissect mice by removing the skin and organs completely. Remove the fat from the mice but be careful that damage is not done to the specimen. Place neonates in 15ml falcon test tubes and older embryos in 50ml test tubes.
2. Fix the specimen in 95% ethanol for 12-48 hours slowly rocking at room temperature. (Older embryos require longer fixation!!) The specimen may be placed in 95% ethanol for long periods of time without any damage.
3. Replace 95% ethanol with Alcian blue staining solution for 1-3 days slowly rocking at room temperature. For vertebral or appendicular skeleton, 1-2 days is enough, whereas, head staining requires 3 days.
4. Replace Alcian blue solution with 95% ethanol for 6 hours slowly rocking at room temperature.
5. Replace 95% ethanol with 2% KOH solution for 12-24 hours slowly rocking at room temperature. Older animals will require more time than younger ones. If you want to remove any remaining fat or skin, do so at this point.
6. Stain specimen in Alizarin Red Solution for 12-24 hours slowly rocking at room temperature.
7. Clear skeleton in 1%KOH/20% glycerol solution until the specimen is completely cleared. Do not leave skeleton in this solution for TOO long because the skeleton will become extremely fragile.
8. Replace clearing solution with 1:1 glycerol:95%ethanol for 1 day.
9. To store skeletons for long periods of time, pass the skeletons through glycerol/ethanol solutions. (50%, 80%, and 100%)

Solutions:

Alcian Blue

0.03% Alcian Blue

80% ethanol

20% acetic acid

Alizarin Red

0.03% Alizarin Red

1% KOH

water