

Skeletal Preparation of Newborn Mice

(Rivera lab)

All steps are done at room temperature in Petri dishes. To avoid disarticulating the skeletons, do not move them replace the solutions.

1. Remove the skin and viscera using forceps. To remove the epidermis from hands and feet, scald the carcass for 30 seconds in 65°C - 70°C water.
2. Place carcass in 6-cm tissue culture plate. If several carcasses are processed at the same time, use 6-well plates to facilitate handling.
3. Fix overnight in 95% ethanol.
4. Stain in Alcian Blue solution overnight.

95% ethanol	800 ml
Acetic Acid	200 ml
Alcian Blue 8GX (Sigma A3157)	150 mg

5. Put back in 95% ethanol for 2-5 hours.
6. Treat with 2% KOH for 24 hours until mostly clear. At this point, remaining fat and skin can be removed easily. If mice were adult, could treat for 2 days or longer.
7. Stain overnight in 1% KOH, 0.015% Alizarin Red (Sigma A3757).
8. Clear skeletons in 1% KOH, 20% glycerol for 2 days or more.
9. If skeletons are not cleared after 2 days, put them back in 1% KOH until they clear or use forceps to remove excess tissue.
10. Store skeletons in a 1:1 mixture of glycerol and 95% ethanol. Document your results, alizarin red staining fades with time.