

Staining of cartilage and bone in adult, fetal or neonatal mice

The key **reference** for this procedure is M. Jean McLeod, *Teratology* **22**: 299-301 (1980).

Adult or neonatal mice are killed using dry ice. They are then skinned, eviscerated extensively, and considerable effort needs to be paid to remove ALL soft tissues. Leaving soft tissue remnants greatly compromises the quality of the data at the end of a month-long procedure!!

1) Fix mice in 95% EtOH for ≥ 5 days

2) Place in standard lab Acetone (neat) for 2 days to remove any residual fat

All the following steps are done in the dark:

3) Stain 3 days in freshly prepared and filtered staining solution (see below) at 37 degC

1 vol 0.1% Alizarian red S (in chemical drawer), in 70% EtOH (filtered)

1 vol 0.3% Alcian blue 8GS (in chemical drawer), in 70% EtOH (filtered)

1 vol acetic acid

17 vol 70% EtOH

4) Wash in doubly distilled water

5) Clearing: Clear in 1% aqueous K-OH for 12-48 hrs

6) Clear for 1 week each (sequentially) in:

(20% glycerol) 1 vol glycerol/4 vol 1% KOH

(50% glycerol) 1 vol glycerol/1 vol 1% KOH

(80% glycerol) 4 vol glycerol/1 vol 1% KOH

Standard glycerol from general stores was used.

7) Store skeletal preparations in 100% glycerol in the dark

8) Photograph as needed