

Decalcification protocol

- Make 14% EDTA solution
 - Add 140 g free acid EDTA to 700 ml distilled H₂O
 - On stir plate in the fume hood, add ammonium hydroxide 30 ml at a time until solution clears (about 90 ml total).
 - Add H₂O to almost 1L. Check pH and adjust with ammonium hydroxide dropwise up to pH 7.2, then adjust final volume to 1L.
- Place fixed/rinsed tissue in at least 15 volumes of 14% EDTA, and change daily or 5x/week (M-F), with mixing
 - this means at least 15 ml for a mouse tibia/femur
 - placing tissue in a screw-top tube on its side, on a rocker works well. We tape tubes into a rack and place the whole thing on its side on the rocker.
- Time of decalcification varies with tissue size, species, etc
 - mouse adult long bones and vertebrae, 10-14 days
 - isolated calvaria, 2-4 days
 - **please contact Crystal to discuss your specifics**
- Rinse in H₂O x 4
- for paraffin embedding, place in 30%, 50%, and 70% ETOH for at least 30 min each prior to submitting to core lab
- for frozen sections, blot tissue dry and freeze in OCT
- **DO NOT** decalcify tissue with EDTA prior to submitting for plastic sectioning
- **There is an additional charge if your tissue is not completely decalcified and we have to perform additional decalcification**

Contact Crystal in the core lab 314-747-6034 with any questions.