SOP 018-02-01
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Washington State University – Spokane

Decalcification of bone using EDTA

I. Purpose: The purpose of this SOP is to provide instructions for properly decalcifying rodent bones for microtomy

II. Materials
   A. EDTA
   B. Sodium Hydroxide
   C. pH meter or pH paper
   D. Hot plate and stir bar
   E. 50 mL conical vials
   F. Shake Plate

III. Methods
   A. Prepare decalcification Solution (14% EDTA pH 7.0)
      1. Dissolve 250g EDTA into 1750 mL diH_2O. EDTA does not go into solution easily, so using a magnetic stir bar and hot plate greatly reduce the time necessary to make this solution
      2. pH the EDTA mixture to 7.0 using Sodium Hydroxide (NaOH), approximately 25 g
      3. Adjust the mixture's volume to 2000 mL
   B. Decalcify tissue.
      1. This protocol has been optimized for rat ankles. Larger samples will require more time to decalcify
      2. Place fixed bone specimens in prelabelled 50 mL conical vials and fill to at least 30 mL with EDTA decalcification solution prepared in part A.
      3. Place conical vials on a shake plate at an angle to increase agitation and prevent the sample from lodging in the bottom of the tube. Agitate gently.
      4. Change the solution daily to prevent saturation of EDTA with calcium
      5. Check decalcification progress using any x-ray producing device. Complete decalcification will result in the bones no longer being visible compared to the surrounding tissue.
      6. Decalcified tissue can be grossed into small segments, moved back into formalin or PBS/Sodium Azide for storage, or processed directly