**Center for Musculoskeletal Research**
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**Histology and Histomorphometry Core**

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# DECALCIFICATION OF BONE

**Use a 20% EDTA/PBS solution, **not** acid decalcification methods or commercial reagents.**

20% EDTA in 1x PBS (1 LITER):

1. In a bottle/container, add 100 mL of **10X PBS** and about 500 mL of **dH20**
2. Add **23 g NaOH (pellets)** to the solution and place on a stirrer, stir until dissolved (Note: the EDTA will not be fully dissolved until pH reaches close to 8.)
3. Add **200 g EDTA disodium salt** to bottle and fill dH2O up to **900 mL.** Stir until mostly dissolved – usually takes several hours
4. Once EDTA is mostly dissolved, add NaOH pellet one a time (usually 2-4 pellets) or **NaOH solution** dropwise and check pH, repeating periodically until EDTA is fully dissolved. Final pH should be 8.

5. Add **distilled $H_2O$** to bring the final volume of the solution up to 1L.

PROCEDURE:

1. Dissect bone and remove as much soft tissue as possible.
2. After appropriate fixation, wash tissue in distilled $H_2O$.
3. Place tissue in 20% EDTA solution (use 20x the sample volume to ensure complete saturation).
4. For best results, change EDTA solution 2-3 times a week.
5. Decalcification is complete when bone is soft and pliable (check with Histo Core if unsure).
6. Rinse 3x with distilled $H_2O$. (Please ensure samples are thoroughly rinsed – excess EDTA can damage our tissue processing machine!)
7. Submit to Histo Core in **70% ethanol.**

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*Please note that decalcification usually takes about 2 weeks at 4°C for normal adult mouse bones, but the time needed will vary based on:

- degree of mineralization
- frequency of solution changes
- size of specimen
- storage temperature during decalcification (reaction will happen faster at room temp than 4°C)
- whether samples are agitated manually or continuously (using a shaker/rocker)