



Histology & Histomorphometry Core

EMAIL: ENDOCRINEHISTOCORE@PARTNERS.ORG

Decalcification of Bone

Use 20% EDTA, don't use acid decalcification methods or commercial reagents.

Making 20% EDTA

EDTA disodium salt, 200 gm,
distilled H₂O, 950 ml,
10N NaOH, ~50ml.

Combine above and stir until EDTA dissolve. Check pH and adjust to 7.4 with NaOH.

Procedure to decalcification bone:

Specimens can be decalcified in EDTA solution over several days up to several weeks in a refrigerator at 4°C with intermittent shaking to make a sure the decal solution is flowing around the bone. The time of decalcification depends on degree of mineralization and size of specimen.

1. Dissect bone and remove as much soft tissue as possible.
2. After appropriate fixation, wash tissue in distilled H₂O.
3. Place tissue in EDTA with shaking or agitation at 4°C. This may take one day to several weeks depending on age of animal and tissue size. Use 20X more volume of EDTA solution to saturate tissue.
4. Change EDTA solution twice or three times a week.
5. Decalcification is complete when bone is soft and pliable. Usual 2 weeks for normal adult 4 weeks old mouse bone. Check with HistoCore if unsure.
6. Rinse with distilled H₂O 3X.
7. Submit to HistoCore in 70% ethanol (ethanol mix with distilled H₂O).