MASSACHUSETTS GENERAL HOSPITAL

Center for Skeletal Research

# Histology & Histomorphometry Core

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## Embedding and sectioning of decalcified frozen sections

1. Always dissect tissue, including bone ,in ice cold buffer (Saline or PBS) to prevent drying and preserve tissue morphology.
Place freshly dissected tissue in 4°C 4% paraformaldehyde in an ice bucket, ASAP after harvesting. Use a fixative volume at least 20X the volume of the tissue.
(To make paraformaldehyde warm up 440 ml distilled H2O to 60°C on a heated stir plate, don't let it get up to 65°C. If it does cool it to 60°C . Weigh 20 g paraforma ldehyde (PFA) in a hood and stir in the prewarmed water on a heated hot plate in the hood. Don't let it get up to 65°C. If it does discard in appropriate chemical waste container and start over. Add ~50 ul 10N NaOH which will help the PFA dissolve. After dissolving PFA, add 50 ml 10X PBS, pH7.4. Check final pH, it should be 7.4 and total volume should be 500 ml. Store in 4 °C overnight or freeze at -20 for up to 2 weeks.)

2. Decalcification (for postnatal bone and other mineralized tissue): proceed as per EDTA decalcification protocol below.

3. After complete decalcification (or if no decalcification is required), rinse in PBS 3X.
- Transfer bone tissue to 30% sucrose/PBS, overnight at 4°C, older bone tissue should be stored 2 -3 days in 30% sucrose/PBS. Incubate in equal volume OCT/30% sucrose for overnight; embed bone in OCT with proper orientation.
- Submit samples to Histocore on dry ice with electronic requisition form.

<u>Decalcification protocol</u>

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

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.

1. After appropriate fixation, wash tissue in distilled H2O.
2. Place tissue in EDTA. Specimens are decalcified in EDTA over several days up to several weeks at 4°C with intermittent shaking to make a sure the solution is flowing around the bone. The decalcification time depends on degree of mineralization and size of specimen. Use 20X more volume of EDTA solution to saturate tissue.
3. Change EDTA solution twice or three times a week.
4. Decalcification is complete when bone is soft and pliable. Usual 2 weeks for normal 4 week old mouse bone. Check with Histocore if unsure.
5. Rinse with distilled H2O 3X.