



Histology & Histomorphometry Core

EMAIL: ENDOCRINEHISTOCORE@PARTNERS.ORG

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 or 4% formalin 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 paraformaldehyde (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
 - Transfer tissue to 50% sucrose/PBS, overnight at 4°C or until it sinks
 - Incubate tissue for 4 hours in 50% sucrose/50% OCT for 4 hours or until it sinks
 - Embed in OCT
 - Submit samples to Histocore on dry ice with electronic requisition form

Mice are injected with calcein, 20 mg/kg

To make up calcein:

0.2g sodium bicarbonate, 0.02g of calcein,

Add 10 mls of dH₂O, stir, then filter sterilize. Can keep for up to 2 weeks wrapped in foil at 4C

Inject 100 mcl per 10 g of body weight intraperitoneally

For mice about 4 weeks old, dose interval 24 hours and sacrifice 24 hours after last fluorochrome injection

For 6 week old mice, dose interval 4 days and sacrifice 48 hours after last injection.

For 10 week old mice, dose interval 1 week and sacrifice 48 -72 hours after last injection.

Carefully dissect bones free of muscle and connective tissue, fix in 70% Ethanol and deliver to the core with a filled out requisition