

Center for Musculoskeletal Research

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Histology and Histomorphometry Core

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Resin processing, embedding and sectioning of non-decalcified mineralized tissue

1. Remove rear legs from the hip joint; be careful to keep the femoral head intact. Cut through the skin and muscle with a scissor until you reach the joint, the femoral head should release itself from the hip joint. Cut off all muscle and remove all skin, except the skin on the paw. Leave the entire leg and paw intact. Take care to not break the fibula. If you do break the fibula, leave the epiphysis intact and keep the fibula attach to the tibia. We need this as landmark for orientation purposes. Separate the femur from the tibia and fibula and immerse the entire leg into cold 70% Ethanol (20-25 ml per bone). We normally save the femurs for microCT, tibia for histomorphometry. If you don't require microCT, you may reserve the femur for other studies (histology, cell isolation, biomechanics, RNA, etc.)

NOTE: Bones can be fixed in 4% PFA or 10% Formalin overnight in the cold before rinsing in PBS and transferring to 70% alcohol, but Alkaline phosphatase staining can only be done on bones fixed directly in 70% alcohol)

Perform this dissection in PBS to avoid drying of the specimen.

2. Turn the mouse over, and from the dorsal side, cut through the skin at the bottom of the spine to reveal the lumbar vertebrae. Cut along the spine to separate whole vertebrae, always pick up L1-L5. Put the vertebral sample into a separate scintillation vial of cold 70% Ethanol (20-25 ml per vertebral specimen). Make sure tissue is always immersed in fixative.

Perform this dissection in PBS to avoid drying of the specimen.

If mice have been injected with fluorochromes, bones should be put into scintillation vials, cover the vial with foil to shield from light.

3. Fix bone overnight at 4°C on a shaker. Label the vials with a pencil or cover the label with transparent tape since ethanol dissolves markers and pens. If the vial leaks, pencil label will stay, other marker labels will disappear.
4. Change cold 70% ethanol daily for total three days until the bone is completely fixed then end the bone to Histocore.
5. If vials require prolonged transportation, pack with ice packs.



6. Fill out electronic requisition form. Make sure tissue vials are all clearly labeled and match to the electronic requisition form.
7. Please include with tissues, their genotypes, birth dates, sex, collection date and any other information you think might be useful.