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Preparation of samples for Osmium Tetroxide Staining

Osmium tetroxide is radiopaque and can be used to stain marrow fat in mouse long bones. The stained marrow fat can subsequently be scanned and quantified using µCT. We ask that you fix your bones using the following protocol (adopted from Scheller *et al.*) and then submit the bones to our core for osmium tetroxide staining and µCT imaging. When you submit your samples we will perform a scan of the mineralized bone for analyzing trabecular and cortical architecture. Following the scan of the mineralized bones, we will decalcify the bones, stain them with osmium tetroxide, and perform a second µCT scan to image the osmium tetroxide staining.

Sample Fixation:

1) Harvest the samples (please email microCTcore@partners.org for a detailed protocol for harvesting mouse bones). Osmium tetroxide will bind to lipids in the soft tissue surrounding bones, so it is important to remove as much of the soft tissue around the bone as possible. Note: we have found that the tibia is the most reliable bone to stain for marrow fat in mice.
2) Place the bones into individual tissue processing cassettes and then place the cassettes into a container of 10% neutral buffered formalin (the volume of formalin should be enough to more than cover the cassettes). Allow the specimens to fix for 24 hours at 4°C with gentle agitation (orbital shaker or rocker). **Note**: <u>It is important not to use organic compound such as ethanol to fix the specimens due to their capacity to solubilize lipids.</u>
3) After 24 hours, pour off the formalin, rinse the bones 1X with cool tap water, and then wash the fixed bones for 1 hours in cool tap water.
4) Transfer the cassettes to phosphate buffered saline (PBS) and store at 4°C until you are ready to bring your samples to the Imaging & Biomechanical Testing Core for analysis.

Reference:

Scheller EL, *et al*. Use of Osmium Tetroxide Staining with Microcomputerized Tomography to Visualize and Quantify Bone Marrow Adipose Tissue in vivo. *Methods Enzymol*. 2014; 537: 123-139.

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