



How to Submit Decalcified or Soft tissue to HBMI Core

HBMI Core Guidelines for Formalin Fixed Paraffin Embedded Tissue (FFPE)

1. Tissue must be dissected well. Remove all skin, fir and as much muscle tissue as possible. When preforming gross dissection be sure to keep your tissue hydrated in 1xPBS.
2. Fix samples in 10% Neutral Buffered Formalin (NBF):
 - a. Postnatal and adult limbs: Fix 3 days/72 h in NBF at room temperature with agitation.
 - b. Human tissue: Fix 3 days/72 hrs in NBF at room temperature with agitation.
 - c. Hind paw/tendon, front paw and ankles: Fix 3 days/72 h in NBF at room temperature with agitation.
 - d. Soft tissue: Fix 3 days/72 h in NBF at room temperature with agitation.
 - e. Embryos (mouse): Fix 1 day/24 hrs in NBF at room temperature with agitation.

*Speak to histology core members for any other tissue type and fixation time.
3. After fixation rinse your samples in 1XPBS 3 times, each for 5 minutes. Then 1, 5 minute rinse in dH2O.
***Ensure all pins are removed from al femur or tibia fractures after rinsing**
4. Bring samples to the HBMI Core in dH2O for decalcification, along with your completed work order forms.

Tissue Washing Protocol

1. Wash tissue 3 times, 5minutes each in 1 X PBS.
2. Wash tissue 3 times, 5 minutes each in ddH2O.
3. Wash tissue in 50% EtOH, 1 time for 15 minutes.
4. Wash tissue in 70% EtOH, 1 time for 15 minutes.
5. Transfer tissue to fresh 70% EtOH, labeled 70% EtOH to process, and put in refrigerator until processing.

*If tissue is not going to be process right away stop rinsing after ddH2O and put back into 10% NBF.

Submit tissue to core with work order.

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