



Protocol for Formalin Fixation of Tissues Prior to Paraffin Processing

1. Dissect postnatal/adult mouse limbs, calvaria, spines, and embryos in fresh, sterile 1XPBS. Prior to fixation all skin/fur and nearly all muscle tissue **MUST** be removed or fixation will be incomplete (see examples below).
2. You will be responsible for fixing your tissues in 10% Neutral Buffered Formalin (NBF) according to the table below:

Postnatal/Adult Mouse Limbs	3 days (72 hours) in 10% NBF
Postnatal/Adult Mouse Spines	3 days (72 hours) in 10% NBF
Postnatal Calvaria	2 days (48 hours) in 10% NBF
Completely Skinned Embryos	16 – 18 hours in 10% NBF

3. Following fixation, wash the tissues 3 times in fresh, sterile 1XPBS for 5-10 min each at room temperature (rocking). At this time you **MUST** remove PINS from all fractured femurs.
4. Label histology cassettes appropriately for each sample and deliver them to the histology core in 1XPBS with your completed work order form. If the samples are not immediately received, they need to be placed in 70% EtOH at 4°C until someone in histology is available.
5. Samples prepared inappropriately will **NOT** be processed by the histology core.

Notes: Calcein double-labeling experiments on adult bones must be performed on plastic sections. These specimens DO NOT get fixed in 10% NBF, but instead are placed directly into 70% EtOH and given immediately to the histology core facility. Specimens should be delivered fully trimmed of muscle before delivery.