

Fixation protocols

Paraffin embedding

- unless specified in other protocols (eg 4% PFA for some IHC), use 10% neutral buffered formalin
- place fresh tissue in 15-30 x tissue volume formalin, at room temp 24-48 hr
 - for example, use at least 15 ml for a mouse tibia/femur
 - fixation up to 72 hr is generally OK, but some stains such as TRAP may be weaker with fixation > 48 hr
- rinse formalin out of tissue in water 3x15 min, with mixing
- for paraffin, go to decal protocol
- for frozens, blot tissue to remove excess water before placing in OCT
 - freeze in dry ice or bring to histo core to use histobath (2-methylbutane)
 - liquid N₂ is not a great choice for freezing for histology
 - store frozen tissue at -80°C until you bring it to the lab (on dry ice)

Plastic (MMA) embedding

- In most cases, tissue can be placed directly in 70% ETOH (adequate in most cases for typical bone stains and calcein labels).
- If you wish to fix your tissue first, use the formalin protocol above, but only fix for 24h, and place tissue in 70% ETOH after rinse step
- **DO NOT** decalcify tissue with EDTA prior to submitting for plastic sectioning

OCT embedding for frozen sections

- You will have to determine for yourself whether or not to fix your tissue before freezing. For many immunostaining protocols, you fix the slides rather than the whole tissue.
- If you decide to fix before freezing, place fresh tissue in 15-30 x tissue volume formalin, at room temp 24-48 hr
 - rinse formalin out of tissue in water 3x15 min, with mixing/shaking
- blot tissue to remove excess water before covering in OCT in plastic mold
 - avoid freezing air bubbles in your blocks
 - freeze in dry ice or bring to histo core to use histobath (2-methylbutane)
 - liquid N₂ is not a great choice for freezing for histology
 - store frozen tissue at -80°C until you bring it to the lab (on dry ice)
- **DO NOT** place tissue in alcohols prior to freezing

Contact Crystal in the core lab 314-747-6034 with any questions.