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Tissue Preparation for Paraffin Embedding

1. Disect desired tissue and immersion fix in 3.75% paraformaldehyde (EM grade), 1X PBS (Mg & Ca free) overnight at 4°C (if left longer than o/n at 4°C, epitopes may be lost)

   -heat PBS (atleast 55°C), add PFA while stirring into solution, 0.4 μm filter, cool to 4°C on ice.

   -this is a convenient state for shipping (FedEx o/n on wet ice), or wait until tissues are embedded (end of step #4)

2. Rinse tissues briefly in 1X Mg- Ca-free PBS

3. Place tissues in embedding cassettes (if tissue is skin, place gauze or small sponge on top of tissues to eliminate curling)

4. Process embedding cassettes through graded alcohols to xylene with constant stirring

   start time
   _____ 30 min 30% ETOH @ RT
   _____ 30 min 50% ETOH @ RT
   _____ 30 min 70% ETOH @ RT **Convenient stopping point; place at 4°C up to several days
   _____ 60 min 80% ETOH @ RT
   _____ 60 min 95% ETOH @ RT
   _____ 60 min 95% ETOH @ RT
   _____ 60 min 100% ETOH @ RT
   _____ 60 min 100% ETOH @ RT
   _____ 60 min 100% ETOH Highest grade
   _____ 45 min Xylene in hood @ RT
   _____ 45 min Xylene in hood @ RT
   _____ 45 min Xylene in hood @ RT
   _____ 60 min 55°C paraffin in vacuum oven (stirring not necessary)
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   _____ Equilibrate cassettes/tissues in embedding paraffin for 30 min if different from processing paraffin

   Embed in paraffin blocks paying attention to orientation, leave border of paraffin around tissues
   Cool blocks slowly to avoid cracking.

5. Cool blocks to -20°C prior to cutting 5μ sections

6. Let sections dry @ RT o/n prior to analysis.