

## Paraffin Wax Embedding Protocol

### ***Tissue fixation***

- Prepare fresh fixative (paraformaldehyde 4% and 2.5% glutaraldehyde in 0.1M phosphate buffer), keep at 4°C.
- Immerse the tissue in fixative and leave overnight at 4°C.

### ***Dehydration of fixed tissue***

- Wash 2x10 min in phosphate buffer 0.1M, pH7.2
- Dehydrate in an ethanol series at room temperature (RT).
  1. 2x10 min EtOH 10%
  2. 2x10 min EtOH 20%
  3. 2x10 min EtOH 30%
  4. 2x10 min EtOH 40%
  5. 2x10 min EtOH 50%
  6. 2x10 min EtOH 60%
  7. 2x10 min EtOH 70%  
(If you need to, you can stop and keep the samples at 4°C overnight or for months or years at -20°C)
  8. 2x30 min EtOH 80% RT
  9. 2x30 min EtOH 90% RT
  10. 2x30 min EtOH 100% RT
  11. 1x40 min EtOH 100% RT

### ***Histoclear clearing agent***

1. 1x1 hr. 25% Histoclear:75% EtOH RT
2. 1x1 hr. 50% Histoclear:50% EtOH RT
3. 1x1 hr. 75% Histoclear:25% EtOH RT
4. 2x1 hr. 100% Histoclear RT

### ***Embedding in Paraffin wax***

- Add 5-6 chips of Paraplast plus to each sample and leave overnight at RT.
- Place samples in the wax oven at 56-58°C to melt wax.
- Add paraplast chips about every half hour until melted. Keep the volume of the solution constant by removing some of the melted paraplast with a warm Pasteur pipette.
- Add more chips until 100% wax. Leave overnight in the oven.
- Melt plenty of wax chips in a beaker and leave in oven.
- Change liquid wax 2x for the next two days.
- Pour fresh melted wax into mould, add sample, orientate and cover with wax.
- Apply stub holder to sample and float mould in ice water.
- Store at 4°C.

***De-waxing sections on Slides***

- Fully immerse slides in the following solutions:
  1. 100% HistoClear: 2 x 2min
  2. 100% Ethanol: 2 x 2min
  3. 70% Ethanol: 1 x 2min
  4. 50% Ethanol: 1 x 2min
  5. 30% Ethanol: 1 x 2min
  
- Rinse with dH<sub>2</sub>O and stain immediately.