EMBEDDING FOR CRYOSECTIONS OF MOUSE TISSUE

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Material

PBS 1x pH 7.4
Paraformaldehyde 4% in PBS 1x pH 7.4 (freshly made)
0,1M phosphate buffer pH 7.4 (see Maniatis)
50mM NH4Cl in PBS 1x pH 7,4
5% sucrose in 0,1M phosphate buffer
20% sucrose in 0,1M phosphate buffer
Tissue-Tek Embedding medium

Protocol

Note. Fixation, washing and cryoprotectant solutions should be in vast excess over the volume of tissue.

1. Rinse tissue/embryoid bodies (EBs) in PBS 1x pH 7.4, 2x 5 minutes, RT°C.
2. Transfer EBs to a 2ml tube.
3. Fix tissue in PFA 4% **(2 hours 4°C for EBs, overnight for embryos > e11.5, and adult tissue)** on the rotator)
4. Remove fixative using a Pasteur pipette (do not touch the EBs). Fill tube with 50mM NH4Cl solution, invert and incubate 5 minutes at RT°C.
5. Wash 2x 10minutes in 0,1M phosphate buffer
6. Incubate 1x 60 minutes at RT°C in 5% sucrose
7. Incubate 1x 60 minutes at RT°C in a 1:1 mix of 5% and 20% sucrose
8. Incubate overnight at 4°C in 20% sucrose
9. Prepare a bath of ethanol 95%/dry ice.
10. Transfer to embedding “cup”. Remove as much solution as possible, without drying the tissue.
11. Fill approx. half of the embedding “cup” with embedding medium.
12. Center the tissue using a P200 tip. Do not make air bubbles.
13. Using forceps, place the bottom half of the “cup” in a dry ice/ethanol bath. Hold the “cup” until all of the embedding medium has frozen (approx. 2-3 minutes).
14. Store the frozen block at -80°C until sectioning.