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JOHNS HOPKINS MEDICINE

Hackam Lab
Frozen Tissue in OCT or Cryo-Gel

Experimental Information

1. Store tissues for cryostat sections at -80°C.
2. Section tissues 5-10 micron thickness, store sections at < -20°C or at -80°C.
3. Warm the slides at RT for 5 minutes.
4. Rehydrate tissue with 1x PBS, two changes with 5 minutes' incubation each time.
5. Mark section edges with Pap Pen (be very precise to save antibodies).
6. Fix tissue with -20°C cold acetone for 5-10 min at RT *(Note: Acetone fixation will also Permeablize the sections)* **or 4% Paraformaldehyde for 10 min only** (*Please be aware*, *fixing in PFA for more than 10–15 min will cross link the proteins to the point where antigen retrieval may be required to ensure the antibody has free access to bind and detect the protein—so avoid over-fixation).*
7. Wash with 0.5% BSA/1xPBS -3 changes (incubate 2–5 minutes each time).
8. **OPTIONAL:** Add 2M HCl incubate at room temperature for 40 minutes (Brdu antigen retrieval only if mice are injected with Brdu). Or go to step 10.
9. Wash with 0.5% BSA/PBS – 3 changes (incubate 2–5 minutes each time).
10. **BLOCKING:** Block nonspecific binding with 1% BSA with 5% goat serum for 60 minutes at room temperature.
11. **PRIMARY ANTIBODIES:** Add primary antibodies (1:200 in 0.5% BSA) incubate overnight at 4°C.
12. Wash with 0.5% BSA/PBS – 3 changes (incubate 2–5 minutes each time).
13. **SECONDARY ANTIBODIES**: Add Fluorescent labeled antibodies (1:1000 in 0.5% BSA) incubate overnight for 60 minutes at room temperature.
14. Remove secondary antibodies and add 200 µL DAPI, incubate for 15 minutes.
15. Wash with 1XPBS – 5 changes (incubate 2–5 minutes each time).
16. Mount using Gelvatol solution.