

Immunofluorescence

Frozen Cryostat Sections

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DAY 1

- Mount tissue in OCT and section in cryostat. Collect 14 micron sections on Fisher SuperFrost Plus slides. Do not let sections dry longer than it takes to collect them.
- Fix immediately after sectioning for 7 minutes in 4% Paraformaldehyde/1X PBS. Paraformaldehyde solution should be fresh (<7 days old)!
- Wash 2 times 10 minutes, 1XPBS. If you are doing a lot of sectioning, fix and transfer to 1XPBS as you go along, then do final 2 times 10 minute washes in 1XPBS.
- Permeabilize 30 minutes in P/T (1XPBS, 0.1% TX-100).
- Transfer slides to humidified chamber outfitted with rails made of 2 mL pipettes. Chamber is humidified by placing strips of paper towels or blotting paper in between the rails and wetting paper with P/T. Blot back of slide with a KimWipe and place onto pipette rails (tissue side up). Block for 30 minutes in P/T/S (1XPBS/0.1% TX-100/5% heat inactivated normal serum, usually goat) by pipetting about 500 ul P/T/S onto each slide.
- Prepare appropriate dilution of primary antibody in P/T/S. Working with one slide at a time, tip slide to remove blocking solution and apply 200 ul to 500 ul of diluted primary antibody per slide. If antibody is precious, apply only 100ul to slide and add a coverslip to inhibit evaporation. **MAKE SURE THAT SECTIONS DO NOT DRY OUT AT ANY TIME!!** Incubate overnight at 4 degrees.

DAY 2

- Wash sections 3 times 10 minutes in P/T.
- Block sections 30 minutes in P/T/S (500 ul per slide), using horizontal method described above. Remove block before adding secondary antibody, as described above.
- Incubate with secondary fluorescent antibody, two hours at 25 degrees in the dark (cover chamber with foil or place in a drawer). Do not exceed two hours. Dilute antibody in P/T/S, 1:800.
- Wash sections 3 times 5 minutes in P/T. Shield from light by covering staining jar with foil or placing jar in a drawer.
- Individually blot excess buffer and mount in 60 ul Vectashield (Vector Labs). Store in the dark at 4 degrees C; fluorescent signal usually fades in 1 to 4 weeks.

TYPICAL PRIMARY

Rabbit Anti-B-Gal	1:800
Rabbit Anti-GFP	1:1000

SECONDARY

Goat Anti-Rabbit CY3	1:800
Goat Anti-Rabbit Alexa 488	1:800