Date:

Slides and tissue sections:

1. Slides are stored at -80°C. Quickly load cold slides into slide rack, then place slide rack in jar containing 4% formaldehyde, 20 min
2. PBS wash, 10 min × 3
3. [OPTIONAL] 1.0% H_2O_2, or 1.0% $NaBH_4$ and 20 mM glycine in PBS, 20 min
4. [OPTIONAL] PBS wash, until bubbles disappear.
5. Remove slides from rack, draw along the slide edges with A-PAP pen, and place on slide staining tray.
6. Blocking: 10% NGS + 1% BSA + 0.3% Triton X-100 in PBS, 1 hr

	Per well	For total of slides
BSA	10 mg	mg
NGS	100 µl	µl
Triton X-100 (3% stock)	100 µl	µl
PBS	800 µl	µl
Total	1000 µl	µl

7. Primary antibodies in PBS containing 1% NGS, 1% BSA, 0.3% Triton X-100, overnight at RT
   a. Ab 1 = anti- (1: = µl/well;)
   b. Ab 2 = anti- (1: = µl/well;)

	Per well	For total of slides
BSA	5 mg	mg
NGS	10 µl	µl
Triton X-100 (3% stock)	100 µl	µl
PBS	890 µl	µl
Total	1000 µl	µl

8. PBS wash, 10 min × 3
9. Secondary antibodies in PBS with 1% BSA, 1% NGS, 0.3% Triton X-100, 1 hr at RT
    a. Ab 1 = anti- (1: = µl/well;)
    b. Ab 2 = anti- (1: = µl/well;)

	Per well	For total of slides
BSA	5 mg	mg
NGS	10 µl	µl
Triton X-100 (3% stock)	100 µl	µl
PBS	890 µl	µl
Total	1000 µl	µl

10. PBS wash, 10 min × 3
11. DAPI, 5 min
    a. stock solution = 1 mg/ml in purified water
    b. working solution = dilute stock solution 1:1,000 in PBS
12. PBS wash, 10 min × 3
13. Saline (0.9% NaCl, aq) wash
14. Apply coverslip with Aqua/Poly anti-fade mountant
    a. List of slides: