Hackam Lab for Pediatric Surgical, Translational and Regenerative Medicine
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JOHNS HOPKINS MEDICINE

Hackam Lab
IHC for Whole Mount

REAGENTS

1. **4% PFA:** Obtain 16% PFA, place into 50cc tube and add PBS to 40 mL.
2. **PBS-PFA-0.1% Triton x 100:** Add Triton x 100 (40 µL) into 40mL of 4% PFA.
3. **10% & 1% Normal Donkey Serum (NDS) in PBST**

METHOD

Day 1

1. Remove the media from chamber slides
2. Wash the slides with PBS (x 1).
3. Place 300 µL of PBS-PFA-0.1% Triton X 100 into each well for 1 hr at RT.
4. Wash with PBS for **15 min** at RT (x 4).
5. Block with 10% NDS in PBST for **45 min** at RT.
6. Incubate with primary antibody in 1% NDS/PBST in 4 degree fridge on rotator **overnight.** Dilution condition of most primary antibodies is **1:250**.

Day 2

7. Wash with PBST on rotator in 4 degree for 4-5 hours changing PBST at least (x 5).
8. Incubate with secondary antibody in 1% NDS/PBST in 4 degree fridge on rotator **overnight.** Dilution condition of secondary antibody is **1:1000**.

Day 3

9. Add Dapi directly to the secondary antibody (add double the amount already within the well) for 15 min.
10. Wash with PBST on rotator in 4 degree for 10 minutes (x 6).
11. Remove the wells with the razor blade and tacking.
12. Place mounting gel without bubbles on each well.

Place coverslip and place into dark, dry shelf.