Protocol for Whole Mount Immunofluorescent Staining

1. Rehydrate
   100% MeOH, 75% MeOH in PBT, 50% MeOH in PBT, 2x PBT (5 min each)
   .2% Triton-X in 1x PBS

2. PBT Wash (2x 15-20 min)

3. Block in 5% bovine serum albumin in PBT (1 hr)

4. 1?Ab(overnight in 4°C)
   Dilute to proper concentration in 5% BSA

5. PBT Wash (4x 15-20 min)
   0.2% Triton in 1x PBS

6. 2?Ab(3 hr)
   Dilute to proper concentration in 5% BSA
   **IN DARK!

7. PBT Wash (4x 15-20 min)
   0.2% Triton in 1x PBS
   Hoechst (1:1000) in wash 3 (30 min)
   **IN DARK!

8. Dissect and mount
   Dissect and position desired sample in PBT
   Cover in anti-fade and place cover slip over sample
   Let dry and set before imaging
   **IN DARK