

## 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<sup>st</sup>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<sup>nd</sup>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