# Zebrafish Whole Mount Immunohistochemistry

**REAGENTS:**

Fixation:
4%PFA
Methanol
75 uM 1-phenyl 2-thiourea (PTU) (if appropriate)
PBST: 50 ml 1X PBS + 50 µl Tween-20

IHC:
Ice cold acetone
DAPI 5:250
PBST
Block: PBS 1X + 0.1% Tween20-20% + 5% Sheep Serum
Primary antibody 1:100 in block (may vary)
Secondary antibody 1:750 in block (may vary)
Rehydration Solutions:
- 95% MeOH + 5% PBST
- 75% MeOH + 25% PBST
- 50% MeOH + 50% PBST
- 25% MeOH + 75% PBST

**PROCEDURE:**

**Fixation:**

1. If embryos are to be fixed later than 24hpf, you may want to rear them in PTU to prevent pigmentation (unless this interferes inherently with your experiment). PTU should be added to E2 medium *prior* to initial onset of pigmentation (28 somites).

2. Fix embryos in 4% PFA at time-points of interest. Place approximately 15 embryos/1 ml 4% PFA in a microfuge tube and rock 3-4 hours at room temperature.

3. Rinse embryos 5x 5 minutes each in PBST.

4. Store in methanol at -20C.

**IHC**:

1. Rehydrate embryos gradually in MeOH/PBST solutions – 5 minutes each:
    a. 95% MeOH + 5% PBST
    b. 75% MeOH + 25% PBST
    c. 50% MeOH + 50% PBST
    d. 25% MeOH +75% PBST

2. Wash at least 4x 5 minutes in 1ml PBST.

3. Permeabilize the embryos in 1ml ice cold acetone for 8 minutes.
4. Wash at least 4x 5 minutes in 1ml PBST.
5. Incubate the embryos twice for 1 hour in 1ml block.
6. Add primary antibody at 1:100 concentration.
7. Incubate 1 day on gentle rocking device at 4°C.
8. Wash embryos 5x 10 minutes in 1ml block .
9. Wash 3x 10 minutes in 1ml PBST.
10. Add appropriate 2° antibody 1:750.
11. Wrap in foil and incubate overnight at 4°C on gentle rocking device.
12. Wash embryos 5x 10 minutes in 1ml block.
13. Wash 3x 10 minutes in 1ml PBST.
14. Apply optional fluorescent stains (e.g. DAPI) x 30 minutes.
15. Remove DAPI, add 1ml block.
16. Mount and view embryos or store at 4°C in block until analysis.