Whole-Mount Antibody Staining of Zebrafish Embryos

Reagents

- Paraformaldehyde
- 10X PBS
- Triton X-100
- Primary antibody
- Alexa-Fluor conjugated secondary antibody
- BSA
- DMSO (optional)
- Methanol
- Normal Sheep Serum (heat inactivated at 56 °C for 30 minutes)
- Methylcellulose
- Glycerol
- Permount (Fisher SP15-100)

Procedure

Fixation and storage of embryos

1) Remove chorions for embryos older than 18 somites.
2) Fix embryos in 4% formaldehyde in PBS 1-2 hours 25°C, or 4-12 hours 4 °C.
3) Dechorionate embryos that are younger than 18 somites.
4) Transfer embryos into 100% methanol and store them at –20 °C (2 hours to several months).

Antibody staining (Day 1)

1) Rehydration. Transfer embryos into microfuge tubes and rehydrate them by successive incubations in the following solutions (25°C, ~750 µL/tube):
   - 75% MeOH/25% PBS for 5 minutes (no agitation)
   - 50% MeOH/50% PBS for 5 minutes (no agitation)
   - 25% MeOH/75% PBS for 5 minutes (no agitation)
   - 100% PBX (PBS containing 0.1-1.0% Triton X-100) for 4 x 5 minutes (rocking agitation; percentage of detergent will depended on antibody and embryo stage used).

2) Blocking. Block in antibody blocking buffer (650 µL/tube; 10% sheep serum, in PBX containing 0.5% BSA, and 1% DMSO (optional)) for several hours at room temperature, or overnight at 4 °C, with rocking agitation.

3) Primary antibody staining. Dilute primary antibody to desired concentration in antibody blocking buffer. Remove blocking buffer from embryos and replace with diluted antibody solution. Incubate overnight at 4 °C with rocking agitation.

Antibody staining (Day 2)

1) Washes at room temperature with rocking agitation:
   - PBX, very brief wash
   - PBX for 5 minutes, 5 times
   - PBX for 20 minutes, 3 times
2) **Blocking.** Block in antibody blocking buffer (650 µL/tube; 10% sheep serum, in PBX containing 0.5% BSA) for 1-2 hours at room temperature with rocking agitation.

3) **Secondary antibody staining.** Dilute secondary antibody 1/200 in antibody blocking buffer. Remove blocking buffer from embryos and replace with diluted antibody solution. Incubate room temperature for 2-3 hours with rocking agitation.

4) Washes at room temperature with rocking agitation:
   - PBX, very brief wash
   - PBX for 5 minutes, 5 times
   - PBX for 20 minutes, 3 times

5) Embryos may then be transferred to a 1:1 PBS/glycerol solution for documentation or mounting.