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 sore 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.