Whole-Mount Embryo labeling with (Monoclonal) Antibodies

Source: Zoltan Varga; this protocol is a derivative of the chapter found in the Zebrafish Book, Westerfield, 2000/ Karen Larison)

Solutions

PO_4 buffer (0.1 M, pH 7.3):

80 ml 0.1 M Na_2HPO_4
(13.8 g $Na_2HPO_4 \cdot H_2O$/Liter ddH_2O)
20 ml 0.1 M NaH_2PO_4
(26.8 g $NaH_2PO_4 \cdot 7H_2O$/Liter ddH_2O)

2X PBS:

8.0 g NaCl
0.2 g KCl
200 ml 0.1 M PO_4 Buffer, pH 7.3
300 ml dH_2O

When diluted 1:1 with dH_2O, final concentrations are 0.8% NaCl, 0.02% KCl and 0.02 M PO_4.

4% Paraformaldehyde (Fixative):

Mix equal amounts of
8% paraformaldehyde and
2x Phosphate buffered saline (PBS, pH 7,3)

PBS/BSA/Triton:

50 ml 2X PBS, pH 7.3
1 g BSA
1 ml 10% Triton X-100
Add dH_2O to a total volume of 100 ml.

Block:
2% Normal goat serum (NGS) in PBS/BSA/Triton

Primary antibody:
2% Normal goat serum (NGS) in PBS/BSA/Triton + 1º Antibody (dilute according to recommendation)

Secondary antibody: (fluorescence, or enzyme tagged)
2% Normal goat serum (NGS) in PBS/BSA/Triton + 2º Antibody (dilute according to manufacturer's recommendations)

Embryos remain in Eppendorf tubes throughout the staining procedure; remove and add solutions as indicated.

1. Fix embryos in 4% paraformaldehyde for 3-4 hours at room temperature or overnight at 4°C.
2. Wash 3x in 0.1 M PO_4 buffer, pH 7.4, for 5 min.

Permeabilization steps 3. – 6. are optional:

3. Wash in dH_2O for 5 min.
4. Freeze in acetone at –20°C for 7 min. to permeabilize tissue.
5. Wash in dH_2O for 5 min.
6. Wash in 0.1 M PO_4 buffer for 5 min.

7. Treat with PBS/BSA/Triton + 2% NGS for 30 min to block non-specific binding sites.
8. Soak in primary antibody (Mouse IgG) diluted in PBS/BSA/Triton + 2% NGS overnight at 4°C. Best in cold room on shaker to facilitate penetration. Shorter incubation times are also possible at room temperature.

9. Wash 4x 30 min in PBS/BSA/Triton at room-temperature.
10. Soak in secondary antibody diluted in PBS/BSA/Triton + 2% NGS overnight at 4°C or 5 hr at RT.
11. Wash 4x 30 min PBS/BSA/Triton (overnight at 4°C).

12. Transfer embryos into PBS.
13. Mount tissue in 4% Methyl cellulose on slide, using pieces of coverslips to serve as spacers. The embryos can also be viewed using depression slides.

Thorough washes/rinses seem to be the most important factor to reduce background (if any) additional or extended wash/rinsing steps can always be included.

Instead of Acetone you can use **ProK** to permeabilize tissue (optional):

14. use PBS buffer, 10 µg/ml (working dilution) ProK in ddH_20. The exposure time will vary depending on the embryonic larval stage, the batch of Prok you use, and whether you have frozen and thawed the ProK stock solution several times. It is recommended to test each batch of ProK on embryos/larvae that you won't need for experiments, then aliquot and freeze your ProK stock at –20 °C.

After ProK treatment

15. rinse at least 3x rapidly in ddH_20,
16. rinse at least 3x 5min in PBS,
17. re-fix samples in 4% PFA for 20 min at RT,
18. rinse 3x 5min in PBS.