Xenopus Whole Mount Immunostaining

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Fix embryos in MEMFA for 2 hours at RT or ON at 4ºC.
Cut embryos in MEM w/o formaldehyde.
Fix in memfa 1hr-30min.
Bleach overnight at RT in dent's and H2O2=2:1. Dent's fixative: DMSO:methanol=1:4

Wash in PBSw at RT 4 X 1h.

Block in PBSw + 3% BSA + 20 % goat serum (blocking solution) for 2 h at 4ºC. Simultaneously, prepare a dilution with the 1º Ab (from 1:200 to 1:200 depending on the antibody and incubate) for 2h at 4ºC.

Remove the blocking solution and add the 1º Ab in blocking solution to the vials containing the embryos and incubate o/n at 4ºC. We use National Scientific Target DP 2ml CERT 4000-93W CLEAR ID VIAL KIT W/BND SLIT T/S, 100PK (VWR cat# 66065-642) in which we use 300-500µl of antibody dilution.

Wash in PBSw at RT 4-5 times at 1hr each.

Mix secondary peroxidase-conjugated antibody to blocking solution and block for 2hr at 4ºC. Simultaneously, add 2º Ab to an aliquot of Blocking solution and incubate for 2h at 4ºC. We use 1:700 for anti-rabbit IgG HRP and 1:500 for anti-mouse IgG HRP.

Remove the blocking solution and add the 2º Ab in Blocking solution to the vials containing the embryos and incubate o/n at 4ºC.

Wash in PBSw 4-5 times for 1hr each.

DAB + Ni solution. DAB=10x (-20ºC) (ROCHE Cat. No. 1718096) + DAB Buffer (4ºC) + $NiCl_2$ (12µl/1ml DAB mixture), Add 500µl to each vial.

Monitor the reaction. Brown signal will often be visible within 1-5 minutes. Generally, no improvement in signal is seen after about 15 minutes.

This nickel enhanced procedure is several fold more sensitive than the regular DAB procedure. While this reaction is more sensitive than the brown HRP reaction, it produces a more granular staining that may make fine details harder to visualize.

Stop the reaction by washing 3 X 20min with PBST.

Keep embryos in ethanol by adding increasing serial dilutions (25%, 50%, 75%, 100%).

Solutions

10X PBS
18.6 mM NaH2PO4 (2.56 g NaH2PO4 . H2O per 1000 ml dH2O)
84.1 mM Na2HPO4 (11.94 g Na2HPO4 per 1000 ml dH2O)
1750.0 mM NaCl (102.2 g NaCl per 1000 ml dH2O)
Adjust pH to 7.4 with NaOH or HCl. Prepare 1X PBS by diluting 1:10 with dH20. Both 1X and 10X PBS can be kept indefinitely at room temp.

PBST
1X PBS
0.1% Triton X-100
Mix 100 ml 10X PBS, 899 ml dH2O, and 1 ml Triton X-100. Store at 4ºC or at room temp.

DAB+Ni solution
Prepare an **8% solution of nickel chloride** (Fisher Cat. No. N54-500) in dH20. This 8% solution can be stored indefinitely at room temperature. Prepare the DAB+Ni solution by combining **1 ml of the 0.3 mg/ml DAB solution described above with 12 µl of 8% nickel chloride**. Mix well and use immediately. It is not advisable to store DAB containing nickel chloride because the nickel will precipitate out of solution (as nickel phosphate) after a few hours.

Example of finished product in a Stage 10.5 embryo using anti-dpERK antibody:

Antibody Staining

Purpose:

Date Run:

By:

Vial #	Stage	1º Ab	1º Ab Conc.	2º Ab	2º Ab Conc.	Notes
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