Xenopus Whole Mount Immunostaining
Protocol 2

Jack Greenan 2012

PROTOCOL:

Day 1

-Collect pigmented embryos in glass vials and fix in 1X MEMFA overnight at room temperature on Model 55 Rocking shaker (reliable scientific inc.).

Day 2

-Wash 3 times 10 min in distilled water.
-Cut embryos in desired orientation with sharp scalpel in distilled water.
-Wash for 10 min each in 25%, 50%, 75% and 100% EtOH series.
-Bleach embryos in mixture of 1/3 Hydrogen peroxide 2/3 EtOH, with the vials placed under direct fluorescent light. Monitor bleaching procedure every 3 hours. If bleaching appears to have stopped add fresh EtOH/Hydrogen peroxide mix. Leave overnight.

Day 3

-Continue bleaching if any pigmentation is still apparent, otherwise continue to next step.
-Wash 3 times 10 min in PBST (1X PBS with 0.1% Tween20).
-Remove all PBST and replace with 10 mM Sodium Citrate pH 6.0. Place vials in 70°C water bath for 3 hours.
-Wash 3 times in PBST then Wash in acetone series in PBST (25%, 50%, 75% and 100%) for 10 min each. Place on Model 55 Rocking shaker. Leave overnight at room temperature.

Day 4

-Wash in acetone series in PBST (75%, 50%, 25%) for 10 min each. Remove all PBST and add fresh WMBS reagent (minimum of 1 ml per vial). Place vials in tube rack right side up and place on Genemate Gyromixer at 4°C for at least 2 hours.
-Remove all WMBS and replace with 500 µl solution of primary antibody diluted in WMBS (usually 1/200 dilution works the best). Incubate overnight on Genemate Gyromixer at 4°C.

Day 5

-Remove primary antibody which can be reused if stored at 4°C for a maximum of 3 weeks. Discard sooner if precipitate starts to form.
-Proceed to a quick wash in PBST and replace with WMBS reagent (no antibody) for 2 hours on Genemate Gyromixer at 4°C.
-Remove WMBS reagent and add 1ml of HRP-conjugated secondary antibody diluted in WMBS (1/1000 is optimum. 1/500 usually gives background). Incubate overnight on Genemate Gyromixer at 4°C. NB: Secondary antibody is aliquoted upon arrival and frozen. Aliquot may only be thawed 3 times.

Day 6

-Remove secondary antibody and replace with WMBS reagent (no antibody) and incubate for 2 hours on Genemate Gyromixer at 4°C. Remove all WMBS and proceed to HRP activity staining using DAB substrate. Staining should appear within 20 minutes.
-Once staining is complete remove DAB, wash 3 times in PBST and dehydrate in EtOH series for long term storage in -20°C.

SOLUTIONS:

WMBS: for 1ml add the following,
685 µl DEPC water
10 µl Tris-HCL 1M pH 7.6.
50 µl DMSO
100 µl animal serum. Serum should be from the same specie as secondary antibody (i.e if antibody was made in rabbit then rabbit serum should be used).
155 µl 1M NaCl

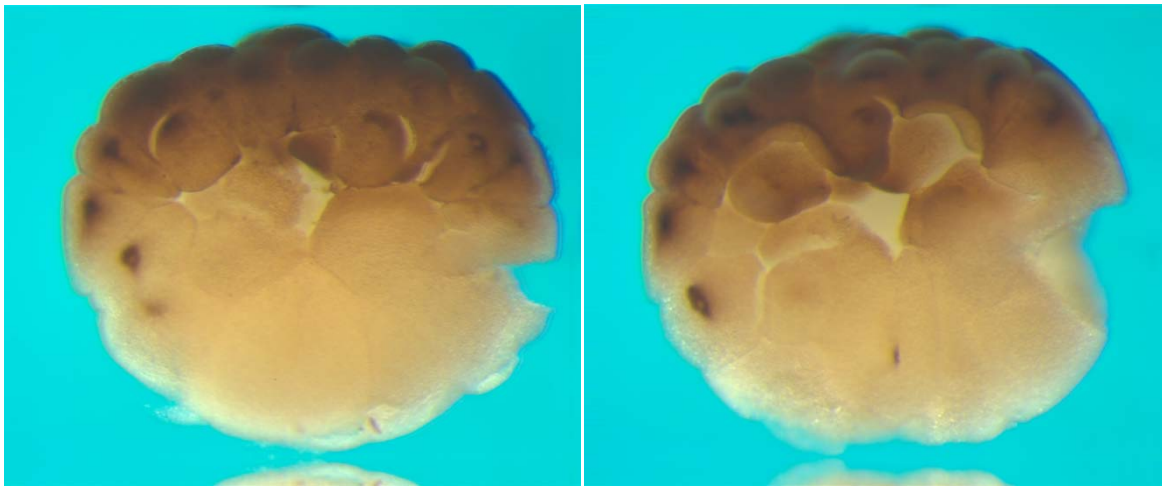
DAB (1ml)
100 µl DAB substrate (Roche cat# 11718096001)
775 µl DAB buffer
127 µl 5% NiCl solution

PBST (1L)
100ml 10X PBS
900ml DEPC water
1ml tween20 (0.1% tween20 final)

Antibodies that have worked

Invitrogen Rabbit Anti-Axin reference 345900

Sigma Chicken Anti-GSK3 reference GW22779



Immunostaining of Axin protein. Embryo was fixed in 1X MEMFA, 3 hours and 30 minutes post fertilization and stained using the above protocol.