

RMO44 Antibody Staining Protocol, Moens Lab

1. At 24 hrs of development (prior to the formation of pigment), change embryo medium. Culture embryos in 0.003 % PTU (phenyl-thio urea) dissolved in Embryo Medium to prevent pigment formation.
2. Dechorionate embryos and allow to straighten (PTU makes chorions somewhat brittle). Fix 30-40 embryos/tube at 48 hr of development in 2% TCA (trichloroacetic acid) made in 1X PBS. Fixation should last 3 hr. at room temp. Embryos will turn white within 5 minutes of initiating fixation.
3. Wash embryos 2 times 5 minutes with 1 ml 1X PB at room temp.
4. Wash embryos 3 times 5 minutes with 1 ml 1X PB-Triton with 0.5% TritonX-100 (PB-Triton).
5. Block non-specific binding sites using 1 ml PB-Triton+ 10% goat serum + 0.1 % BSA (Blocking Solution) for 1 hr at room temperature.
6. Add 0.3 ml per tube of Antibody. Either:
 - a) 1:1 mix of unused RMO44 (in Cecilia Ab box at 4°C) with Blocking Soln –OR-
 - b) reuse aliquot of RMO44 (in Andrew Ab box at 4°C). Check to make sure old RMO44 is NOT cloudy. Antibody should be able to be reused 5-10 times (note tick marks on top of tube). Incubate with RMO44 overnight at 4°C.
7. Remove RMO44 Antibody and SAVE, marking number of uses on top of tube.
8. Wash embryos 6 times 15 minutes each with PB-Triton.
9. Dilute Secondary Ab (Goat anti mouse-HRP conjugated) 1:200 in Blocking Solution. Add to 0.3 ml/tube embryos and incubate at room temp. 5 hrs. at room temp. OR overnight
10. Wash embryos 6 times 15 minutes each with PB-Triton.
11. Wash 2 times with PBS-0.5% DMSO.
12. Dilute Tyramide 1/100 in Diluent. Tyramide/diluent located in NEN kit which is in 4°C deli case. Develop 3 minutes at room temp with approx 0.3 ml dilute tyramide per tube.
13. Wash 4x 10 min with PB-Triton. Clear by washing 10 minutes in 30% glycerol/Tris then 50% glycerol/Tris.
14. Store in dark in deli case. Deyolk and score on fluorescent microscope.

PB = 0.1 M phosphate buffer pH 7.4

PB-Triton = PB with 0.5% Triton-X-100