

Acetylated Tubulin and RMO44 immunostaining

1. Dechorionate embryos and fix 10-15 embryos/tube 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.
2. Wash embryos 2 times 5 minutes with 1 ml 1X PB at room temp.
3. Wash embryos 3 times 5 minutes with 1 ml 1X PB-Triton with 0.5% TritonX-100 (PB-Triton).
4. Block non-specific binding sites using 1 ml PB-Triton+ 10% goat serum + 0.1 % BSA (Blocking Solution) for 2 hr at room temperature or ON at 4°C.
5. Add primary Ab in blocking solution:
RMO44 1:50
Acetylated tubulin 1:1000
ON at 4°C
6. Wash embryos 6 times 15 minutes each with PB-Triton.
7. Add secondary Ab (Alexa Fluor 488) 1:500 in Blocking Solution and incubate ON at 4°C.
8. Wash embryos 12 times 15 minutes each with PB-Triton.
9. Put embryos in glycerol and image.

PB = 0.1 M phosphate buffer pH 7.4

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