

Wholemount immunocytochemistry for RyR.

All the solutions are kept and used at 4oC. Incubations and washes are performed at 4oC with gentle agitation on a flat bed rotary shaker.

- 1) Dissect tissue in PBS (Sigma) and transfer tissue to a 1.5 ml eppendorf tube.
- 2) Fix tissues in 100% Methanol for 5 minutes at -20oC
- 3) Wash 3 times for 10 min. in PBS.
- 4) Fix again in PBS containing 2% Paraformaldehyde + 0.1% Glutaraldehyde for 20 min.
- 5) Wash 3 times for 10 min. in PBS.
- 6) Wash 5 times or more over a period of 2 hours in PBT (PBS, 0.1% Triton X-100).
- 7) Block in PAT (0.5% BSA, 5% Goat serum, 0.1%Triton in PBS) for 1 to 2 hours.

Pablo's tubule wholemount ICC for ER proteins

Written by Administrator

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8) Add affinity-purified RyR antibody diluted 1:100 in PAT and incubate overnight at 4°C.

9) Wash all day in PBT changing solution every hour.

10) Block in PAT for 1 to 2 hours.

11) Add secondary antibody (Jackson Immunologicals: Texas red-conjugated affinity-purified goat anti-rabbit antibody diluted 1:500 in PAT), cover tubes with silver foil and incubate overnight at 4°C.

12) Wash 3 times for 20 min in PBT (Go to step 6 if more than 1 antibody is going to be used)

13) Wash 4 times for 1 hour in PBS (All day if you can gives better results)

14) Optional. Incubate with DAPI and 3 x5 min washes with PBS.

14) Mount slides. We use Vectashield mounting medium.

Good luck!!