Tardigrade Embryo Immunostaining Protocol

By Willow Gabriel, for Hypsibius dujardini embryos

• use siliconized eppendorf tubes for all steps
• wash by centrifugation at 4500 rpm for 3 min, followed by aspiration with a Pasteur pipet
• carry out all steps with agitation
• day 1 = 3.5-4 hours, after embryo collection; day 2 = 4.5-5 hours

1. Treat with chitinase (5 units/ml)/chymotrypsin (10-20 mg/ml) in 0.5x PBS for 1 hour.
2. Wash 3X - 5 min in bottled spring water (we have used Deer Park and Crystal Geyser).
3. Fix in ice-cold absolute methanol for 20 min at 4°C.
4. 90% MeOH for 5 min at RT.
5. 70% MeOH for 5 min at RT.
6. 50% MeOH for 5 min at RT.
7. Fix for 10 min in 4% PFA in 0.5X PBT (w/ 0.05% Triton, not Tween) = 250µl 16% EM grade PFA + 750µl 0.5X PBT (Cat. # 15710, EM Sciences, www.emsdiasum.com)
8. Sonicate on a Branson 250 sonifier for 20 sec (5 sec pulses, 15 sec on ice between pulses) at an amplitude of ~2.2 with a constant duty cycle.
9. Allow to recover on ice for 15 min.
10. Wash 5X - 5 min in 0.5X PBT (w/ 0.05% Triton, not Tween).
11. Block 15 min in 5% DMSO, 1% BSA in 0.5X PBT.
12. Add 1° antibody to tube (1:200 for tubulin, e.g.), and agitate in cold room 0/N or at RT for 2 hours (depends on antibody).
13. Wash 3X - 5 min, then 2X - 20 min with 0.5X PBT.
   • All following steps should be carried out in dark.
14. Add 2° antibody (1:200 for Alexa 488 G-M) in 0.5X PBT +5% DMSO → 45 min at RT.
15. Wash 2X - 20 min, 3X - 5 min in 0.5X PBT;
16. tyramide signal amplification (Molecular Probes) - 20 min (+ 2 µl of tyramide/100µl of 1:2000 dilution of H2O2)
17. wash 3X - 5 min
18. DAPI (1µg/ml solution diluted 1:200, final concentration) + topro-3 (2µl per 1 ml 0.5X PBT) for 20 min
19. wash 3X - 5 min
20. Mount on subbed slides with mounting media. Seal with nail polish.