Crustacean antibody staining[1]

INTRO

This protocol can be used for practially any tissue. Animal-specific modifications will all have to do (more or less) with permeability of tissue, which are issues which each person will have to deal with on their own, going by their criteria based on knowledge of the embryo/tissue to be stained.

The first three steps assume that you have already fixed the tissue. You have to do this however you think is best for your particular animal. Bear in mind that fixations appropriate for *in situ* hybridisation are often too harsh for anitbody staining to work well – you want to fix the minimum amount possible for the tissue, particularly embryonic tissue.

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1. Transfer directly from methanol (after fixing or from frozen storage) to PBS with some detergent (e.g. PBS 1x, Triton X-100 0.1%)[2]. Fixation was in 4% paraformaldehyde in PBS 1x at room temperature for 30 minutes. An equal volume of MeOH was added to help animals sink to bottom of tube (50ml falcon). 3-4 washes of 5-10 minutes each in fresh MeOH were done. Then transferred directly to PBS 1x + TX-100 0.1%.
2. Make sure to do all these steps in fairly large volumes: increasing volumes with increasing toughness of outer coating. We did *Artemia* in 4 ml (polystyrene tubes with snap caps), *Mysida* in 2 ml (eppendorfs).
3. If cuticle is tough, **sonicate first**[3]. I sonicated **3 times 3 seconds** (counting slowly) at **50% power** of upstairs sonicator (in IMBB across from Thireos lab), then **1 second** (turn machine on and off again as fast as possible) on **maximum power**. This is an emprical procedure - do it for a few seconds at a time until it looks like the surface has a few debris particles (bits of the animals broken off by sonication). Take note of volume of tube/animals (say 15 ml in a 50 ml falcon), time of sonication, power of machine, and results of staining, for future reference. Too much sonication will result in patchy, irregular background; too little will result in totally white, unstained animals, not even any background seen. Note that you will have to do this every time for a new machine. What a pain.
4. If no sonication is neccesary (like for early stages of *Parhyale* embryos with membranes hand dissected off), proceed to wash[4] (about 1h) and block (about

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[1] The blue notes are what I did that time that the staining on *Artemia fransiscana* with *vasa* worked really well. The animals that I stained were between 6 days and 18 days old and staining was good for all ages.

[2] Michalis says he does not bother rehydrating (or dehydrating) in graded alcohol series; neither does Nipam Patel. He does not wait for them to come to room temperature after -20 storage either, just straight from the freezer into RT PBS + TX100. Any morphological deformations are apparently negligible.

[3] **Always sonicate** for *Artemia*. This is because cuticle secretion apparently begins upon hatching. For *Mysida*, sonication is only necessary for later larval stages, when cuticle has begun to form. Early embryos should be permeable enough with just the formaldehyde fixation and subsequent detergent washes.

[4] If they are coming out of methanol deep freeze, make sure to wash well to remove all traces of

1h)[5]. Washes can be in PBS 1x ,Triton X-100 0.1%, BSA 0.1%. Blocking in same with NGS 3%[6]. Washes were for 3 times 30 minutes, block 1h, at room temperature.

5. Primary overnight 4° C (two nights is fine also, depends on the antibody). I did one night.
6. Wash and block as for primary. Long washes can greatly reduce background but may wash away primary antigen (this has never actually happened to me but Michalis says he has heard of it with some antibodies – yikes!). I washed 4 times 30 minutes at 4 degrees and then overnight in fresh wash solution. This was not on purpose – I left the lab and had forgotten that the animals were still washing!
7. Secondary overnight 4° C (or 2h room temperature). I blocked for 1h at RT with NGS and then incubated overnight at 4 degrees.
8. Develop antibody with appropriate enzymatic reaction (or not for fluorescently conjugated secondaries). I used AP and the signal took about 10 minutes to begin to appear; I left the reaction for about 20-30 minutes total and then washed in PBS 1x plus 10mM EDTA to stop the reaction.
9. Wash in PBS, transfer to Glycerol 50% in PBS 1x and mount in same[7] (for enzymatic reaction-developed stains[8] - for fluoresent preps use appropriate anti-fading mounting medium[9]). Almost three months later after storage at 4 degrees in 70% glycerol staining is still OK.

[5] Time of wash/block should be at least about 30 min, but will depend on hurriedness of stainer.
[6] These are the solutions used by Michalis Averof.
[7] Or let sit in glycerol overnight at 4 degrees, change to 70% glycerol and sit overnight in the fridge again, then mount. The longer the samples clear in glycerol the better your pictures will be - background is reduced and signal contrast is enhanced.
[8] If glycerol is good quality, these preps should last for years if sealed with nail varnish.
[9] However, note that for crustaceans with often unfamiliar morphology, enzymatic staining is often preferable for simultaneous nomarski viewing of tissues and better identification of tissue morphologies. Save fluorescence for fancy quadruple stainings (also remember, fluorescent secondaries