TO STAIN SECTIONS WITH ANTIBODIES

We have some sea urchin embryo sections already mounted on slides. We can stain the sea urchin embryos with anti sea urchin fraction 1-8 antibodies made in rabbits. These are the 8 primary antibodies.

The secondary antibodies are: goat anti-rabbit-alkaline phosphatase. Remember to record exactly what you do at each step, if different from writeup.

1. Deparaffinize the tissue by dipping the slides in 100% xylene several changes for 2 min total., then 100% and 50% isoprop for about three more total with a gentle agitation. 22 degrees or colder. Shake off the excess liquid.
2. Place the slides in a TBS wash bath for further rehydration for 5 min.
3. BLOCKING STEP. Drip onto the slides into TBS-5%BSA or type of serum (goat) of secondary antibody for 15 min AT 37 DEGREES. Keep in moist environment (place slide in a petri dish with wet filter paper and place in incubator. Remove the excess TBS-BSA by blotting around the tissue.
4. **CAREFUL HERE, YOU NEED A CONTROL WHICH WILL BE DIFFERENT ONLY AT THIS STEP- ONE SLIDE WILL HAVE THE PRIMARY ANTIBODY, THE OTHER WILL HAVE control serum**
Wipe around the tissue section and add 150 ul of the diluted primary antibody and place in moist chamber at 35 degrees for 30 min. Use 1/50 and 1/500
5.Rinse dropwise with TBS, then put about 200ul of TBS on top of the section, letting it soak for about 5 min.
Repeat this step two more times.
Allow the slides to drain, shake off the excess fluid and carefully wipe each slide as before.
. So from here on you have 18 slides to be treated identically after this step.

6. Apply enough drops of fast red substrates mixture to cover the section. Incubate 5-10 min. or until desired. Be sure to monitor the subtrate under a microscope, and terminate
the reaction before generalized backgroud stain appears.
To teminate the reaction simply rinse the slides with distilled water from a wash bottle.

r. To Mount the sections use an aquaseous mounting medium such as glycerol. coverslip the section and seal
it with clear nail polish.

In the reference of ATPase detection, used cryosectioning instead of passing through alcohols etc. Kaskgarian, M. and Biemesderfer, D. Preparation and use of monclonal antibodies to Na+,K+ATPase. In Methods in Enzymology 156:392-413. 1988.

SOLUTIONS:

TRIS BUFFERED SALINE
.9% SALINE
TRIS 0.6% or 50 mM Ph 7.5

Other References:

1. Erickson, PA, Lewis, GP, Fisher, SK. 1993 Postembedding Immunocytoichemical techniques for light and electron microscopy. In: Antibodies in cell biology. Ed. DJ Asai. Academic Press, NY.

2. Lojda, Z. Gossrau, R., Scheibler, TH. 1979. Enzyme histochemistry. A laboratory Manual. p46.

3. Pollard, K., Lunny, D., Holgate, C.S., Jackson, P., and Bird, C.C. 1987. Fixation and immunochemical reagent effects on preservation of T-lymphocyte surface membrane antigens in paraffin-embedded tissue. J. Histochem. Cytochem. 35:1329-38.