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41810002 Protocol

Immunocytochemistry/Immunofluorescence protocol for tag-192 Antibody (41810002)

Immunocytochemistry/Immunofluorescence Protocol for tag-192 Antibody (41810002):
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Methanol/Acetone Fixation and Immunofluorescence Staining
of Worm Tissues and Embryos
Strome Lab, MCD Biology, UCSC

Fixation:

1. Use slides treated with gelatin/chrom alum "subbing" solution containing 1 mg/ml polylysine (boxes are labeled GCP slides) to promote sticking of sample to slides. (Note that GC slides are not sticky.)

2. Either place a 10-12 ul drop of eggs (prepared by hypochlorite treatment of worms) on a slide or pick 10-15 worms into a 10-12 ul drop of M9 buffer on a slide and cut them in half to extrude tissues and release eggs.

3. Overlay the sample with an 18 X 18 coverslip. To flatten the specimen, wick excess liquid from the edge of the coverslip, monitoring flattening in the microscope. It is important to flatten the pieces of worm tissue and embryos enough (to promote sticking to the slide and permeabilization of the sample) but not too much (which extrudes cells from the eggshell of embryos and disrupts nuclei in tissues).

4. Immerse the slide in liquid N2 or freeze it on dry ice.

5. Pry the coverslip off with a razor blade, and immediately (before the sample thaws) fix the specimen by placing the frozen slide in a coplin jar containing 4 degrees Celsius methanol (with a layer of molecular sieves in the bottom to make the methanol anhydrous) for 10 min. followed by 10 min. in a coplin jar of 4 degrees Celsius acetone. Alternatively 20 min. of cold methanol may be used.

6. Either air dry the slide or gently dip it into dH2O and then wash at least 30 min. in PBS. We usually air dry.

7. Dry slides are stored at room temperature; they may be stored for weeks depending on the antigen. Slides in PBS are stored at 4 degrees Celsius

Staining:

1. Block by pipetting onto the sample -40 ul of 1.5% ovalbumin, 1.5% BSA in PBS (OV/BSA). Incubate for 30 min. at room temp.

2. Wick off OV/BSA (do not let sample dry), and replace with -40 ul 1 degree antibody diluted in PBS. Incubate overnight at 4 degrees Celsius in a humid chamber (so that the drop of Ab solution does not evaporate).

3. Wash for 3 x 10 min in PBS in a coplin jar at room temp.

4. Block in OV/BSA for 5-10 min. at room temp.

5. Incubate with -40 ul 2 degrees antibody diluted in PBS for 2 hr at room temp in a humid chamber.

6. Wash for 5 x 10 min in PBS in a coplin jar at room temp. Include DAPI (0.5 ug/ml) in the 3rd wash.

7. Mount in Gelutol or Elvanol mounting medium.

Notes:

1. Getting worms and embryos to stick to slides turns out to be one of the major problems associated with fixation protocols. Several factors promote sticking.

a. The age of the poly-lysine-treated slides seems to affect the ability of the slides to retain specimens. Fresh slides are better.

b. Flattening specimens helps stick them onto the slides.

c. Air drying also greatly improves the chances that specimens will stick to the slides. Many of the specimens fall off the slides when they are transferred from acetone to dH2O to PBS.

2. Methanol/acetone has proven to be the fixative of choice for immunofluorescence visualization of a variety of antigens. Methanol/acetone fixes by coaggulating proteins; it also extracts tissues, resulting in relatively low background staining. However, because of this extraction, certain antigens may not be retained by the fixation procedure. Also different antigens show differential sensitivity to air drying after acetone and to the length of time slides with fixed specimens are stored before being stained. These various parameters must be determined empirically for each antigen.

3. In addition to fixing tissues and embryos, methanol/acetone renders them permeable to antibodies.

4. Intact L1, L2, L3, and sometimes L4 worms are also efficiently fixed and permeabilized. It is especially important to flatten intact worms before fixing.