

## Immunostaining:

Fix cells as desired (downloadable paraformaldehyde and methanol fixation protocols available on our website). Permeabilize with 1% Triton X-100 in PBS for 15 minutes at room temperature. Block by incubating cells for 10 min in blocking buffer (see below). We perform all blocking and antibody incubation steps in a humidified chamber (fancy name for a box with a lid and a water-saturated tissue in it...line the bottom with parafilm to put the coverslips on).

Incubate cells with primary antibodies diluted in blocking buffer for 35mins to 1 hour. If you have never used the antibody before, try several dilutions (low of 1:100 to high of 1:1000, for example). Wash coverslips 3 X 10 minute with PBS (can do washes in 6-well plates, then transfer back to humidified chamber for secondary antibody incubation). Incubate coverslips for 35mins to 1 hour with the appropriate secondary antibodies diluted in blocking buffer, and wash 3 X 10 minutes with PBS. We use fluorophore-conjugated secondary antibodies from Jackson Immunochemicals.

If desired, stain DNA with DAPI (1:15,000 dilution in water of a 5 mg/ml stock). A good RNA stain, which fluoresces in the red channel, is Pyronin Y (1:10,000 dilution in water of a 10 mM stock; must stain with DAPI first). Wash well with PBS and mount cells as desired (we use either MOWIOL/Dabco, which hardens, or the liquid mounting media Vectashield from Vector Labs, in which case the coverslips must be sealed with nail varnish). Slides can be stored in the fridge or freezer until analyzed by microscopy.

### Blocking Buffer (also used to prepare all antibody dilutions)

PBS

1% serum

0.1% Tween-20

*Note: Appropriate serum must be chosen for blocking. Try to use serum from the animal used to raise the secondary antibody (Scottish Antibody Production Unit sells various animal seras).*