

Tissue whole mount immunostaining.v1

Part 1. **Immunostaining** Note: if your tissue is already fluorescently labeled, go to part 2.

1. Harvest desired tissue and incubate 1 mL 4% PFA for 20 minutes (at room temperature) and then transfer to 1 mL PBS. For whole mount and confocal imaging, the tissue needs to be thin or deformable, such as the diaphragm, spinotrapezius, or fat. The tissue can be stored for a few days in the refrigerator. (Optional: you can fix the tissue with formaldehyde prior to this step.)
2. Put the tissue in PBS with 0.2% Saponin overnight in 4°C. This permeabilizes the tissue.
3. Block for 1 hour at room temperature with PBS containing 5% mouse serum, any other serum you need/desire, 0.5% BSA, and 0.2% Saponin. This will be referred to as blocking solution.
4. Add your primary antibodies to the appropriate volume of blocking solution. Bathe the tissue in the antibodies overnight.
5. Wash the tissue 5 times (20 minutes each wash, room temperature) using PBS with 0.2% Saponin. If you used pre-conjugated primary antibodies, proceed to step 2.
6. Add secondary antibody to blocking solution. Place the tissue in the secondary solution for 2 hours at room temperature.
7. Repeat step 5.

Part 2. **Whole mount** This procedure is designed to get a tissue ready for confocal microscopy

Note: We perform all the next steps in a darkened room. We turn off all the lights except for a small lamp.

1. Place the tissue on a gelatin coated slide. Use small paint brushes to orientate the tissue. Make sure there are no air bubbles under the tissue.
2. Allow the tissue to adhere to the slide. This typically takes 5-10 minutes, but it can vary on the thickness and type of tissue
3. Cover the tissue with 50:50 PBS:Glycerol and place a coverslip over the top. You want the coverslip to be as close to the slide as possible. This is easy for thin tissues, and you can push the coverslip and slide together if the tissue is deformable.
4. Remove any excess 50:50 that has come out from under the coverslip.
5. Seal the cover slip by painting the edges with nail polish. This will prevent the 50:50 from evaporating over time.
6. Image immediately.