

Whole mount immunohistochemistry

Everything is performed at room temperature except antibody incubations at 4-10 °C.

1. **Dissect and Fix:** Dissect in 1X PBS and fix in 4% paraformaldehyde, 1X PBS for 20 minutes (or longer depending on your antibody) at room temperature.

With 20 % paraformaldehyde stock:

40 ul 20% paraformaldehyde *

20 ul 10X PBS

140 ul water

With 32 % paraformaldehyde stock:

25 ul 32% paraformaldehyde *

20 ul 10X PBS

155 ul water

2. **Wash:**

A) Wash 3 times in 1X PBS for 15 minutes each. You can store your tissues in PBS at 4°C after this until you are ready to stain. Since we do not use preservatives do not store too long (2 days are usually OK).

B) Wash 3 times in 0.1 M Tris-HCl/0.3 M NaCl (pH 7.4) with 0.5% Triton X-100 (TNT) for 15 minutes each.

3. **Block and Wash:**

A) Block in Invitrogen/molecular probes solution called Image-iT FX signal enhancer (Cat #, 136933) for 30 minutes at room temperature (not longer – very important).

B) Was 3X for 15 minutes in TNT.

4. **Primary Antibody:** Apply primary antibody at right dilution and incubate overnight at 4-10 °C. (dsx 1:100)

5. **Wash:** Wash 6 times in 0.1 M Tris-HCL/0.3 M NaCl (pH 7.4) with 0.5% Triton X-100 (TNT) for 15 minutes each.

6. **Secondary Antibody:** Apply secondary antibody at right dilution (1:200) and incubate overnight at 4-10 °C. For all subsequent steps, make sure that experiment is covered, as secondary fluorescent antibodies are light sensitive.

7. **Wash:** Wash 6 times in 0.1 M Tris-HCl/0.3 M NaCl (pH 7.4) with 0.5% Triton X-100 (TNT) for 15 minutes each.

8. **Mount:** Mount in FluormountG.

Can store mounted tissue at 4-10 °C for a few weeks in dark and still see signal (perhaps longer). It is best to image immediately (within a day or so of finishing the procedure).

Perform dissections in thin-well slides and do all subsequent steps in 3-well deeper dishes (should hold about 400 ul volume, but we typically fill with 200 ul volume for antibody and wash steps).

Recipe for 50 mL of TNT:

37.5 mL	H ₂ O
5 mL	1 M Tris pH 7.5
5 mL	3 M NaCl
2.5 mL	10% Triton X-100

* Purchase EM grade paraformaldehyde that is a premade 20% solution (or 32%); Electron Microscopy Sciences phone is 215-646-1566 (Cat # 15713).