

Immunostaining (Brain)

Fly brains (up to 20-30 brains per line, depending on your dissection skills)

PBS

PBST-lite (0.1% TritonX in PBS)

PBST (0.3% TritonX in PBS)

PFA solution (900µl PBST, 100µl formaldehyde) – Use EM quality liquid 20% PFA

Blocking solution (10% Normal Goat Serum in PBST)

Antibody solution (5% Normal Goat Serum in PBST)

Primary antibody solution (Antibody diluted in Antibody solution)

Secondary antibody solution (Antibody diluted in Antibody solution) (spin Ab before use!)

Anti-fade solution (Vectrashield)

1. Dissect flies in **PBST-lite** and store brains in PBS on ice.
2. Incubate in 300 µl **Formaldehyde solution** for 20 minutes at room temperature to fix tissue.
3. Wash in 500 µl **PBST**, 3 X 10-15 minutes. The longer the better.
4. Block in 300 µl **Blocking solution** for at least 2 hours at room temperature.
5. Incubate in 300 µl **Primary antibody solution** for 24-48 (depends on Ab) hours at 4°C.
6. Wash in 500 µl **PBST**, 3 X 10-15 minutes at room temperature. Wash overnight at 4°C.
7. Incubate in 300 µl **Secondary antibody solution** (spin down for 5 minutes before use) for 12-24 (penetration) hours at 4°C.
8. Wash in 500 µl **PBST**, 3 X 10-15 minutes at room temperature. Wash overnight at 4°C.
9. Wash again for a few hours. Wash for 5-10 minutes in 500 µl PBS before mounting.
10. Mount on slide with small drop of **Anti-fade solution** for microscopy.

Antibody

Rabbit α-GFP polyAb (Torri Pines)

Mouse NC82 for neuropil

Fluorophore conjugated Secondary antibody

Dilution

1:3000

1:20

1:500

Tricks:

For the incubations: make sure you use some detergent in the primary antibody solution, provided that this will not decrease immunoreactivity. Long incubation times are better than short ones. If you need to stain deeper in the brain, you can go up to 48 hours incubation at 4C. Don't pin down the tissues. Rather leave them free floating in the ab solution for the entire staining time.

For the washes: Make sure you use something black to put under the multi well that you are going to use for washes, that would help. Then lean the wells towards you and suck gently with a p200 pipette. Discard the wash solution in a well next to the one you are washing, this way you will not discard the brains accidentally. A bit of practice and you should be ok.