



For a video of dissection of larval CNS see “Larval Dissection” at <https://www.janelia.org/project-team/flylight/protocols>.

Dissection

- Select a larva of the appropriate stage and dissect the CNS in cold phosphate buffered saline 1X (PBS):
 - Using brain pickers, remove a wandering third instar larva from the fly vial.
 - Place larva in a dissecting dish full of PBS. Using a pair of dissecting scissors, cut the larva about 1/3 down from the head.
 - After cutting, the nerves exiting the CNS should be visible as a bundle of threads. Using your forceps, grasp the nerves and use them to ground the tissue and keep it from floating away.
 - You may see the back of the VNC attached to the threads. Cut and pull perpendicularly from where you are holding with your forceps. This should reveal the lobes of the brain. Cut away any large pieces of tissue as well as the imaginal discs that are attached to the back of the brain lobes.
- Transfer the dissected tissue immediately to a 2mL Protein LoBind tube with ~1.9 mL of cold 4% paraformaldehyde (PFA) in PBS. Keep the tube of 4% PFA with tissue on ice until the timed fixation begins.
 - Replace your puddle of PBS with fresh cold PBS when it becomes littered with dissection debris.
 - Tissue can be kept in cold 4% PFA for up to 2 hours before timed fixation begins.

Fixation – 4% PFA in PBS 1X for 1 h at RT

- Timed Fixation.** Within 2 hours of dissection, start timed fixation at room temperature (RT) for 1 hour while nutating. Protect samples from light.
- Fix Removal - Washes.** Place the tubes upright to allow the tissue to sink. Aspirate the fixative and fill the tube with phosphate buffered saline with 1% Triton X-100 (PBT) and nutate for 15 minutes at RT. Repeat for a total of 4 washes. Protect samples from light during washes.
- Storage.** Store the tubes of tissue in 1% PBT at 4°C. Nutate or lay the tubes flat in a covered box on a rotator. Protect from light.
 - Typically, these tubes will begin the IHC (immunohistochemistry) process the following day but can be stored for up to 3 days. If stored more than one overnight, aspirate the old PBT and do a brief wash with 1% PBT before beginning IHC processing.