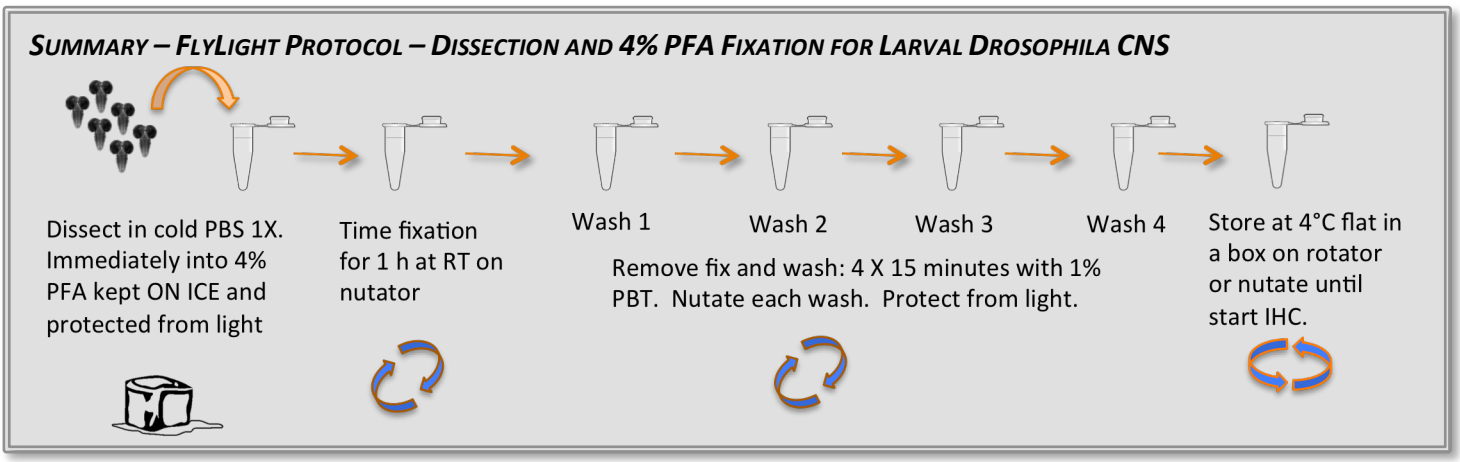

For a video of dissection of larval CNS see Larval Dissection.

Dissection

1. Select a larva of the appropriate stage and dissect the CNS in cold phosphate buffered saline 1X (PBS).
2. 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

3. **Timed Fixation**. Within 2 hours of dissection, start timed fixation at room temperature (RT) for 1 hour while nutating. Protect samples from light.
4. **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.
5. **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.