



For videos of dissection of adult brains see *Adult Brain Dissection* or for adult CNS see *Adult CNS Dissection*.

## Dissection

### 1. De-wax Anesthetized Flies.

- Prepare three wells in a glass well plate: one with 70% ethanol and the other two with cold Schneider's Insect Medium (S2). Place a sticky dot next to the 70% well to clearly distinguish it from the other wells filled with S2.
- Anesthetize flies with CO<sub>2</sub> or cold. Grasp a fly by the wings or legs with forceps and briefly submerge the anesthetized fly first in 70% ethanol (2 seconds) followed immediately by a brief dip in the first well of cold S2. Then submerge the fly in the second well of cold S2 where it will remain until dissected. The ethanol dip removes the wax on the cuticle, helping the fly stay submerged during dissection.
- Do not use a transfer pipette to move flies between wells because this adds ethanol to the S2 wells. Extended exposure to ethanol will accelerate denaturation of the tissue and fluorescent proteins making the tissue unusable.
- Keep the dish with flies submerged in S2 on ice until they are dissected.
- *Do not anesthetize and rinse more flies than can be dissected within 20 minutes.* Flies kept submerged longer will die and their brains will be unusable due to postmortem changes.

### 2. Dissect in cold S2. Transfer the rinsed fly to a Sylgard-lined dish with cold S2 and dissect.

- Replace your puddle of S2 with fresh cold S2 when it becomes littered with dissection debris.
- Dissect only as many flies as you can comfortably complete in **20 minutes** before transferring the dissected brains to fixative. All fixations are precisely timed (see next).

## Fixation – 1.2% PFA in S2 for 24±2h at 4°C

- 3. Timed Fixation.** Within 20 minutes of dissection, transfer tissue to a 2mL Protein LoBind tube with ~1.9 mL of 1.2% **cold** paraformaldehyde (PFA) in S2. Keep 1.2% PFA on ice.
  - Incubate for 24±2 hours at 4°C while nutating. Protect samples from light.

4. **Fix Removal - Rinse.** Place the tubes upright on ice to allow the tissue to sink. Use a transfer pipette to aspirate the cold fix and fill the tube with cold phosphate buffered saline with 5% Triton X-100 (PBT). Invert the tube a few times. Place the tube back on ice and let the tissue settle.
  - Fix should be removed from tubes when they are on ice to ensure the tissues remain at the bottom of the tube during aspiration. If the tube warms up, the air trapped in the trachea will expand causing the tissue to float and possibly be lost during fix aspiration.
5. **Fix Removal - Washes.** Add 1.75 mL of cold PBT and nutate for 10-15 minutes at 4°C. Repeat for a total of 4 washes. Protect samples from light during washes.
6. **Storage.** Store the tubes of tissue in 0.5% 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 overnight, aspirate the old PBT and do a brief wash with 0.5% PBT before beginning IHC processing.