Protocol for GABA staining of Drosophila brains
(larval/adult)

1. Dissect out the brains quickly in fixative (4% formaldehyde in 1X PBS, pH 7.4) on ice. (Take the adult fly/ larva into fixative solution, and take the brain out).
2. Fix the brains in 4% formaldehyde for 1 hr at 4°C.
3. Wash the samples in blocking solution containing 0.1% Triton X-100 (PTX) and 0.1% bovine serum albumin (BSA) in 1X PBS, for 1hr at 4°C on orbital shaker (4 washes, each wash for 15 minutes).
4. Incubate in primary antibody at 4°C on orbital shaker for 2 days.
5. Wash the samples in PBS containing 0.1% Triton X-100 (PTX) for 1 hr (4 X 15 minutes).
6. Add secondary antibody diluted in 0.1% PTX and incubate overnight at 4°C (or 2 hrs at room temperature) in dark on orbital shaker.
7. Wash the samples in PBS containing 0.1% Triton X-100 (PTX) for 1 hr (4 X 15 minutes).
8. Mount the tissues in Vectashield.