

# ***Aedes aegypti* Adult Brain Immunostaining Protocol**

adapted from Riabinina et al., 2016. DOI: 10.1038/ncomms13010

## **Solutions:**

- Fixative: 4% Paraformaldehyde, 0.1M Millonig's Phosphate Buffer (pH 7.4), 0.25% Triton
- PBS: Phosphate Buffered Saline without Calcium or Magnesium
- PBT: PBS containing 0.25% Triton X-100
- Permeabilization Buffer: PBS, 4% Triton, 2% Normal Goat Serum
- Antibody Buffer: PBS, 0.25% Triton, 2% Normal Goat Serum

## **Day 1:**

- Anesthetize mosquitoes on ice and carefully remove heads of adult mosquitoes from the body by pinching at the neck with sharp forceps. Place heads in a 1.5mL tube containing Fixative on ice. Nutate at 4°C for 3 hrs.
- Dissect the brain out of the head capsule in ice cold PBS.
- Wash in PBT for 15 minutes at room temperature. Repeat for 6 washes.
- Permeabilize with PBS, 4% Triton, 2% NGS for 48 hours at 4°C.

## **Day 3:**

- Wash in PBT for 15 minutes at room temperature. Repeat for 6 washes.
- Add primary antibodies in Antibody Buffer for 48 hours at 4°C. The reference brain was generated with anti-NC82 (DSHB 1:50).

## **Day 5:**

- Wash in PBT for 15 minutes at room temperature. Repeat for 6 washes.
- Add secondary antibodies in Antibody Buffer for 48 hours at 4°C. The reference brain was generated with anti-mouse-488 (Invitrogen A11029; 1:500).

### **Day 7:**

- Wash in PBT for 15 minutes at room temperature. Repeat for 6 washes.
- Add Vectashield and let sit 12 hours at 4°C.

### **Day 8:**

- Mount on slides in fresh Vectashield. The coverslip should be elevated 150um to avoid distorting the tissue.