

Free fatty acid (FFA) Assay (Wako kit# NEFA-HR(2))

1. Homogenize 10 larvae in 200 μ L PBS. Keep on ice.
2. Add 100 μ L of reconstituted R1 to each well of the 96-well plate.
3. Add 5 μ L of standard (0, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1 mM FFA), or 5 μ L of homogenate to each well containing Color Reagent A; mix with pipette tip.
 - a. Standard curve: make standards in PBS (freeze at -20C).
4. Incubate the 96-well plate for 10' at 37°C.
5. Add 50 μ L of reconstituted Color Reagent B to each well; mix with pipette tip.
6. Incubate plate for 10' at 37°C.
7. Spin for 5' at 2000x RPM.
8. Transfer 100 μ L to a fresh row or microplate. Avoid disrupting sediment at well bottom.
9. Read at 562 nm in microplate spectrometer.