

## **Fat Body Lipid Stain with Nile Red**

### Prep

Make 4% paraformaldehyde in PBS. Heat at 65-70°C to dissolve. Use within 2 weeks.

Make 10% Nile Red solution in DMF and protect from light with foil.

Dilute 10% Nile Red stock to 0.002% working stock in PBS + 0.1% Triton X-100 (or PBS + 0.1% Tween-20). Protect from light with foil.

### Staining

Dissect FB from 5-10 larvae and transfer to eppendorf tube.

Spin down and remove supernatant.

Add 200 ul 4% paraformaldehyde in PBS and incubate for 20 min at room temp spinning or shaking tubes.

Spin down and dispose of paraformaldehyde in hood.

Wash with 500 ul PBS 3-4X ~5 min / wash. Spin to sediment FB after each wash.

Add 200 ul 0.002% Nile Red in PBS-Triton X-100 for 30 min.

Wash with PBS 3-4X, 5 min / wash.

Mount with VectaShield mounting media with DAPI.

Excitation: 440-500 nm; Emission: >528 nm

OR

Excitation: 515-560 nm; Emission: >590 nm (preferred)

Nile Red: Sigma, N3013-100MG