Oil Red O Staining of Fixed Worms
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[Abstract] Oil red O staining is used to assess major fat stores in *C. elegans*. This protocol is adapted from the Ashrafi Lab at the University of California-San Francisco.

**Materials and Reagents**

1. 20% paraformaldehyde
2. Na$_2$EGTA
3. Spermidine 3 HCl (1 g) (Sigma-Aldrich, catalog number: 85578)
4. Spermine (5 g) (Sigma-Aldrich, catalog number: 85590)
5. Oil Red O (Sigma-Aldrich, catalog number: O0625)
6. NaPIPES (pH 7.4)
7. Beta-ME
8. Isopropanol
9. DTT
10. Tris Base
11. HCl
12. PBS
13. Agarose
14. NaCl
15. KCl
16. Na$_2$HPO$_4$·7H$_2$O
17. KH$_2$PO$_4$
18. NaOH
19. 10x PBS buffer (see Recipes)
20. 1 M Tris-Cl (pH 7.4) (see Recipes)
21. 2x MRWB (see Recipes)
22. Reduction buffer (see Recipes)

**Equipment**

1. Eppendorf Thermomixer Shaker (Eppendorf, catalog number: EF4283A)
2. Dissecting stereo microscope (LEICA MZ12)
3. Compound microscope (Nikon ECLIPSE, model: E600)
4. Tabletop centrifuge
5. 15 ml conical tube
6. 1.5-ml Eppendorf tube
7. 0.2 µm filter

Procedure

1. Collect worms at desired stage with 1x PBS and transfer to 15 ml conical tube. Allow the worms to settle by gravity if using adults or spin down larval stage worms at 1,000 x g for 30 sec.
   Note: Make sure to collect enough worms (~200-500) some will be lost during the procedure.
2. Aspirate supernatant and wash again with 10 ml 1x PBS. Allow to settle by gravity or spin as described at step 1.
3. After second wash, discard most of the supernatant except 400 µl and transfer it to 1.5-ml Eppendorf tube.
4. For fixation, add 500 µl 2x MRWB (freshly made) and 100 µl 20% paraformaldehyde to the tube containing 400 µl sample in 1x PBS.
5. Mix the solution by gently inverting the tube and fix 30 min at RT with gently shaking (1,000 rpm) on an Eppendorf Thermomixer Shaker (invert the tube every 5-8 min without shaking).
6. Wash twice with 1 ml Tris-Cl buffer (100 mM, pH 7.4). Between wash, spin down worms using a tabletop centrifuge at 1,500 x g for 30 sec.
7. After 2nd wash, discard supernatant except of 100 µl and add 900 µl of reduction buffer. Mix by gently inverting the tube and reduce 30 min at RT with gently shaking.
8. Pellet worms by centrifuging at 1,500 x g for 30 sec.
9. Wash with 500 µl 1x PBS; spin down at 1,500 x g for 30 sec.
10. Aspirate to 300 µl, add 700 µl isopropanol. Mix by gently inverting the tube and leave at RT 15 min with gentle shaking.
11. Spin at 1,500 x g for 30 sec to collect.
12. Suck off all isopropanol and add Oil Red O dye solution.
13. Preparation of Oil Red O solution, 0.5 g Oil Red O in 100 ml anhydrous isopropanol, Equilibrate it for two days by stirring at RT.
14. About 15 min before use, mix 4 vol ddH2O with 6 vol dye solution and leave at RT for 15 min. It appears cloudy.
15. Filter the solution with 0.2 µm pore size filter unit. Effluent should appear clear.
16. Add 1 ml of the 60% filtered dye to the worms. Leave on Thermomixer Shaker (700 rpm shaking or a rotator) overnight at RT.
17. Spin at 1,200 x g for 30 sec to pellet worms.
18. Such off as much dye as possible and wash once with 1x PBS.
19. Mount directly on a glass slide with an agarose pad (dissolve 2% agarose in 1x PBS).  
*Note: Keep slides in a humidity container to prevent samples for drying out.*

**Recipes**

1. 500 ml 10x PBS buffer  
   40 g NaCl  
   1 g KCl  
   5.75 g Na₂HPO₄·7H₂O  
   1 g KH₂PO₄  
   Dissolve in 400 ml ddH₂O and adjust to pH 7.4 with HCl or NaOH. Bring volume to 500 ml with ddH₂O.

2. 100 ml 1 M Tris-Cl (pH 7.4)  
   Dissolve 12.114 g Tris Base in 80 ml ddH₂O and adjust to pH 7.4 with concentrated HCl. Bring volume to 100 ml with ddH₂O.

3. 2x MRWB  
   160 mM KCl  
   40 mM NaCl  
   14 mM Na₂EGTA  
   1 mM Spermidine HCl  
   0.4 mM Spermine  
   30 mM NaPIPES (pH 7.4)  
   0.2% beta-ME

4. Reduction buffer  
   100 mM Tris-Cl (pH 7.4)  
   10 mM DTT (1.54 mg in 1 ml)

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This protocol is adapted from the Ashrafi Lab at the University of California San Francisco.

**References**