



Oil Red O Histochemistry for *frozen sections only*

Reagents:

- Oil Red O (Sigma Cat. # O-0625)
- Isopropanol (Fisher Scientific Cat. # A451-4)
- Mayer's Hematoxylin (Sigma Cat. # MHS80)
- 10% Neutral Buffered Formalin (Fisher Scientific Cat. # SF94)
- Vectamount Aqueous Mounting Medium (Vector Labs Cat. # H-5501)

Preparation of Reagents:

- Stock Oil Red O solution (*make this at least a day or two prior to use*)
 - Saturated Oil Red O in 99% Isopropanol (300mg of Oil Red O in /100mL isopropanol)
- Working Oil Red O solution
 - 30 mL of stock solution mixed with 20 mL of Distilled Water
 - Mix and let stand for 10 minutes.
 - Filter prior to use (*takes about an hour to filter properly*)
 - Use Whatman Paper (Cat# 1002-185)
- 60% isopropanol, diluted with MilliQ water

Staining Method:

1. Air dry frozen sections on slides for 30 minutes minimum
2. Fix in 10% neutral buffered formalin for 10 minutes
3. Dip in 60% isopropanol 1 time quickly
4. Stain in working Oil Red O solution for 15 minutes
5. Dip in 60% isopropanol 1 time quickly
6. Dip in DI water 1 time quickly
7. Counterstain with Mayer's Hematoxylin for 3 minutes
8. DI water 10 dips
9. Coverslip with aqueous mounting gel
 - a. IMPORTANT: Do not press on coverslips to remove air bubbles -> Lipids may move!
 - b. ALSO: Do not let air dry. Coverslip immediately after DI water.

Images: frozen sections of mouse liver stained with H&E (left panel) or Oil Red O (right panel)

