

Oil Red O Histochemistry (for frozen sections only)

Reagents

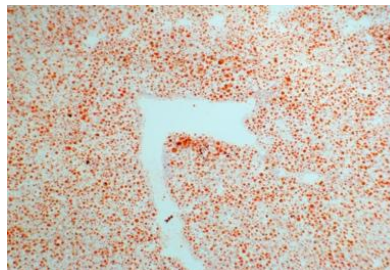
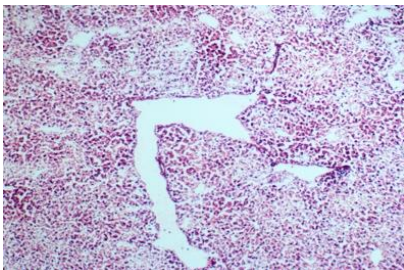
- Oil Red O (Sigma, cat. #O-0625)
- Isopropanol (Fisher Scientific, cat. #A451-4)
- Mayer's Hematoxylin (Sigma, cat. #MHS80)
- 10% Neutral-Buffered Formalin (Fisher Scientific, cat. #SF94)
- Vectamount Aqueous Mounting Medium (Vector Labs, cat. #H-5501)

Preparation of Reagents

1. Stock Oil Red O Solution in 99% isopropanol (*prepare at least 1-2 days prior to use*)
 - a. 300 mg of Oil Red O in 100 mL isopropanol
2. Working Oil Red O Solution
 - a. 30 mL of stock solution in 20 mL distilled H₂O
 - b. Mix and let stand for 10 min
 - c. Filter with Grade 2 Qualitative Filter Paper (Whatman, cat. #1002-185) prior to use (*note: this step takes about 1 h to complete*)
3. 60% Isopropanol Solution (diluted with Milli-Q water)

Procedure

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



Frozen sections of mouse liver stained with H&E (left) or Oil Red O (right)