DAUGHERTY LAB
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Protocols\Pathologies\Histology\
Oil-Red-O Staining

STAINING FROZEN TISSUE WITH OIL RED-O

REAGENTS

1. Saturated stock solution of oil red-O (0.25 - 0.5%) in isopropyl alcohol. Dilute 6 ml of stock solution oil red-O with 4 ml dH_20, let stand for 5 -10 min. Filter the diluted oil red-O (solution only good for 1-2 hours).
2. 0.2 micron syringe filter apparatus or filter paper.
3. 60% isopropyl alcohol made in dH_20 (30 ml isopropyl alcohol + 20 ml dH_20).
4. 4% paraformaldehyde. 2 grams in 50 ml PBS (only good for 1-2 weeks). Alternatively, 10 % neutrally buffered formalin (Fisher).
5. Glycerol gelatin (Sigma).

PROCEDURE

1. Prepare fresh oil red-O.
2. Fix section in 4% paraformaldehyde/PBS or 10 % formalin for 5 min at room temp. Blot.
3. 60% isopropyl alcohol for 5 min at room temp. Blot.
4. Stain with filtered oil red-O for 10 min at room temp. Blot.
5. 60% isopropyl alcohol for 2 min at room temp. Blot.
6. Rinse once with dH_20. Blot.
7. Stain with Hematoxylin for 10 sec. Blot.
8. Rinse with automation buffer 3 - 4 times.
9. Rinse once with dH_20.
10. Mount a cover-slip onto the slide with warmed glycerol gelatin.

REFERENCE

Culling C F A, Allison R T, Barr W T (1985) Cellular Pathology Technique. Butterworth & Co. Ltd., No. 4.

Protocol Developed **:** Alan Daugherty
Updated **:** Hong Lu on 7/18/14

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