油红染色实验报告

一、实验器材及试剂

1、 实验器材

名称	厂家	型号
冰冻切片机	Thermo	CRYOSTAR NX50
切片刀	上海徕卡仪器有限公司	LEICA 819
载玻片	Wanwu	

2、 主要实验试剂

试剂名称	厂家	货号
固定液	Wanwu	G1101
油红染液	Wanwu	G1016
苏木素染液	Wanwu	G1004
分化液（以 60%乙醇为溶剂）	Wanwu	G1039
返蓝液	Wanwu	G1040
甘油明胶封片剂	Wanwu	G1402
异丙醇	国药集团化学试剂有限公司	80109218

二、实验步骤

1、新鲜冰冻切片固定：将冰冻切片复温干燥，固定液中固定 15min，自来水洗，晾干。

2、**油红染色：**切片入油红染液浸染 8-10min (加盖避光)。

3、背景分化：取出切片，停留 3s 后依次浸入两缸 60%异丙醇分化，各 3s、5s。切片依次浸入 2 缸纯水中浸洗，各 10s。

4、苏木素染色：取出切片，停留 3s 后浸入苏木素复染 3-5min，3 缸纯水浸洗，各 5s、10s、30s。分化液（以 60%酒精为溶剂）分化 2-8s，2 缸蒸馏水洗各 10s，返蓝液返蓝 1s，将切片轻轻浸入 2 缸自来水中浸洗，各 5s、10s，镜检染色效果。

5、封片：甘油明胶封片剂封片。

6、显微镜镜检，图像采集分析。

三、结果判读：

脂滴呈橘红色至鲜红色，细胞核蓝色。

四、注意事项：

1、如果是新鲜组织冰冻切片，需要先固定再染色；如果组织是固定以后冰冻切片，可以将切片晾干以后，直接染色。

2、整个操作过程中注意动作轻缓，不宜动作太大，以免脂肪丢失或者移位。

3、染色结果不能长期保存，封片后应尽快观察及拍照。

4、甘油明胶常温下为凝固状，一般 60℃烤箱中保存。封片后如有气泡不能按压玻片也不宜强行扯下盖玻片，玻片可入温水中，让盖玻片自行脱落，然后重新封片，以防脂肪移位。

Oil Red staining report

1 Apparatus and reagents

1.1 Major apparatus

Name	Producer	Model
Frozen slicer	Thermo	CRYOSTAR NX50
Slicer	LEICA	LEICA 819
Glass slide	Wanwu	

1.2 Major reagents

Name	Producer	Code
Fixative	Wanwu	G1101
Oil Red solution	Wanwu	G1016
Hematoxylin solution	Wanwu	G1004
Differentiation solution (With 60% ethanol as solvent)	Wanwu	G1039
Scott Tap Bluing	Wanwu	G1040
Glycerin gelatin sealed tablets	Wanwu	G1402
Isopropanol	SCRC	80109218

2 Procedure

2.1 Reheat and dry the frozen slices, then fix it in the fixative solution for 15 minutes, wash with tap water, and dry.

2.2 Stain sections with Oil Red solution for 8-10 min in the dark, and cover it with lid during dyeing.

2.3 Take out the slices, stay for 3s and then immerse them in two cup of 60% isopropanol for differentiation in turn, 3s and 5s respectively. The slices were immersed to 2 cup of pure water in turn for 10s each.

2.4 Take out the slices, immerse in hematoxylin for 3-5 min after 3s, and then rinse in 3 cup of pure water for 5s, 10s, and 30s in turn. Treat it with differentiation solution (60% alcohol as solvent) for 2-8s, 2 cup of distilled water for 10s each, and Scott Tap Bluing for 1s. Then lightly dip the slices in 2 cylinders of tap water for 5s and 10s in turn, and check the staining effect by microscope.

2.5 Seal the slices with glycerin gelatin.

2.6 Observe with microscope inspection, image acquisition and analysis.

3 Results

Color	Result
Orange/Red	Lipid droplets

Blue	Nuclei

4 Precautions

4.1 If it is a fresh tissue frozen section, it needs to be fixed before staining; if the tissue is fixed after freezing section, you shoud stain after the slices are dried.

4.2 It shoud be gentle during the entire operation process avoid fat loss or displacement.

4.3 The staining results cannot be stored for a long time, and observation and photographing should be done as soon as possible after sealing.

4.4 Glycerin gelatin is solidified at room temperature, so generally stored it in an oven at 60℃. It is also not suitable to press the slide or pull off the coverslip, if there are bubbles after seal the slice. You can put it into warm water to let the coverslip fall off by itself, and then resealed to prevent fat displacement.