V02

油红 O 染色试剂盒（细胞专用）

货号：G1262
规格：4×20mL/4×50mL
保存：2-8℃，避光保存，有效期为 6 个月。

产品组成：

名称		4×20mL	4×50mL	保存
试剂(A):油红O固定液		20mL	50mL	室温，避光
试剂(B):油红O染色液	B1：油红O染色A液	12mL	30mL	2-8℃，避光
	B2：油红O染色B液	8mL	20mL	室温
按B1:B2=3:2比例混合静置10min，即为油红O染色液，不宜提前配制，过滤之后再使用。				
试剂(C): Mayer苏木素染色液		20mL	50mL	2-8℃，避光
试剂(D):油红O缓冲液		20mL	50mL	室温

产品介绍：

中性脂肪(Neutral fat)染色传统方法采用苏丹染料，最近发现偶氮染料油红 O 更适合脂肪的染色。常用染色方法有苏丹II、苏丹III、苏丹IV、苏丹黑 B、油红 O 法等。油红 O 是很强的脂溶剂和染脂剂，较易与甘油三脂结合呈小脂滴状，与磷脂结合力稍差，其染色原理一般认为是物理上的相似相溶作用或吸附作用，利用染料在细胞内脂质溶解率大于染液溶剂中溶解率来使脂肪染色。

油红 O 染色试剂盒（细胞专用）简称 ORO 染色液，可显示最小的脂滴，可优先为脂类从溶剂中吸附染料。标本不采用含有乙醇的固定液，如需要固定可采用 10%福尔马林)。脂肪阳性染色结果呈橘黄至红色，但具体颜色因脂质浓度而定。

自备材料：

60%异丙醇、蒸馏水

操作步骤：(*仅供参考*)

一、培养细胞

1. 移除细胞培养基，用PBS洗两次，加油红O固定液固定20-30min。
2. 弃去固定液，用蒸馏水洗2次，加入60%异丙醇浸洗20-30s。
3. 弃去60%异丙醇后加入新配制好的油红O染色液，浸染10-20min。
4. 弃去染色液，60%异丙醇漂洗10-20s至间质清晰。蒸馏水洗2-5次，直到无多余染液脱出。
5. 加入Mayer苏木素染色液，复染核1-2min。弃去染液后水洗2-5次，加入油红O缓冲液孵育返蓝1min，然后弃去。
6. 加入蒸馏水覆盖细胞并在显微镜下观察。

二、细胞涂片

1. 制备新鲜骨髓、血液涂片，滴加油红O固定液固定10-15min，取出涂片，空气中晾干10-15min。
2. 入新配制好的油红O染色液，浸染15min。入60%异丙醇漂洗10-20s，流水冲洗，入蒸馏水稍微清洗。
3. 滴加Mayer苏木素染色液，复染核2min，入油红O缓冲液孵育返蓝1min，镜下观察染色结果。

染色结果：

中性脂肪	橙红色或橘红色
磷脂	粉红色
细胞核	蓝色

注意事项：

1. 油红O染色工作液不稳定，易产生沉淀，不宜提前配制。
2. 试剂B2在低温储存和运输时会变白色浑浊属于正常现象，沸水浴5min即可恢复澄清正常使用，期间实验人员注意做好防护，避免爆管引起液体飞溅烫伤身体部位。
3. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

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Oil Red O Stain Kit, For Cultured Cells

Cat: G1262
Size: 4×20mL/4×50mL
Storage: 2-8℃, avoid light, valid for 6 months.

Kit Components

Reagent		4×20mL	4×50mL	Storage
Reagent(A): ORO Fixative		20mL	50mL	RT, avoid light
Reagent(B): ORO Stain	B1: ORO Stain A	12mL	30mL	2-8℃, avoid light
	B2: ORO Stain B	8mL	20mL	RT
Mix B1 with B2 as the radio of 3:2 and place for 10 min to form ORO Stain, filter it before use.				
Reagent(C): Mayer Hematoxylin Solution		20mL	50mL	2-8℃, avoid light
Reagent(D): ORO Buffer		20mL	50mL	RT

Introduction

Neutral fat stains often use Sudan II, Sudan III, Sudan IV, Sudan black B, Oil red O and so on. Sudan dye is often used in traditional methods. Recently, azo dye oil red O is more suitable for dyeing fat. Oil Red O is a strong lipid solvent and dye. It is easy to bind to triglycerides in droplet shape, but has a slightly poor binding to phospholipids. The dyeing principle is generally regarded as a physical miscibility or adsorption, and the fat is dyed by miscibility. The solubility of dyes in frozen sections is higher than that in the original solvent, so when dyeing, the dyes are transferred from organic solvents to lipids and the fats are dyed.

Oil Red O Stain Kit, For Cultured Cells is shorten as ORO Stain and can show least fatty droplet. The sample should not fixed with ethanol (10% formalin if required). The positive staining result of fat is orange to red, but the specific color depended on the concentration of lipid.

Self Provided Materials

60% isopropanol, Distilled water

Protocol(*for reference only*)

For Cultured Cells

1. Remove the cell culture medium, wash it twice with PBS, and fix it with ORO Fixative for 20-30min.
2. Discard the ORO Fixative and wash it twice with distilled water. Add 60% isopropanol and soak for 20-30s.
3. After discarding 60% isopropanol, soak in newly prepared ORO Stain for 10-20min.
4. Discard the staining solution and rinse with 60% isopropanol for 10-20s until the stroma is clear. Wash with water 2-5 times until there is no excess dye.
5. Add Mayer Hematoxylin Solution and counterstain the nucleus for 1-2min. After discarding the dye, wash it with distilled water for 2-5 times. Add oil red O buffer for 1min and discard it.
6. Cover the cells with distilled water and observe under the microscope.

For Cell Smear

1. Prepare fresh bone marrow and blood smear, then fix in ORO Fixative for 10-15 min. Take out the smear and put it in the air for 10-15 min.
2. Soak the smear in ORO Stain and dye for 15 min. Rinse with 60% isopropanol for 20-30s, rinse with running water, and wash slightly with distilled water.
3. Re-dyeing the nucleus with Mayer Hematoxylin Solution for 1-2 min. Soak in ORO Buffer for 1min and observed under a microscope..

Result

Neutral Fat	Orange or Red
Phospholipid	Pink Red
Nucleus	Blue

Note

1. ORO Stain is not stable enough and easy to precipitate, so it is not suitable to prepare it in advance.
2. Reagent B2 may turn white and turbid during low-temperature storage and transportation, which is a normal phenomenon. Boiling water for 5 minutes can restore clarity and normal use.
3. For your safety and health, please wear experimental clothes and disposable gloves.