V02

改良油红 O 染色试剂盒(环保型)

货号： G1263
规格： 4×50mL
保存： 室温，避光保存，有效期为 1 年。

产品组成：

名称	4×50mL	保存
试剂(A):油红O染色缓冲液	50mL	室温
试剂(B):油红O染色液	50mL	室温，避光
试剂(C):油红O染色分化液	50mL	室温
试剂(D): Mayer苏木素染色液	50mL	室温，避光

产品介绍：

脂质(Lipid)是中性脂肪、类脂及其衍生物的总称，其共同的物理特性是不溶于水，易溶于有机溶剂(如乙醇、丙酮等)。组织内脂质经常采用苏丹II、苏丹III、苏丹IV、苏丹黑 B、油红 O 法等进行染色。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。油红 O 是很强的染脂剂，较易与中性脂肪结合，与磷脂结合力稍差，其染色原理一般认为是相似相溶作用或物理吸附作用使脂肪染色。染料在细胞内脂质的溶解度较原溶剂中的溶解度更大，所以在染色时染料就从有机溶剂转移入脂质而使脂肪染色。

改良油红 O 染色试剂盒(环保型)使用无味低挥发的环保溶剂替代具有一定危险性的经典溶剂，在不影响脂质定位和大小的情况下大幅降低使用风险和操作难度，对实验操作人员十分友好。脂肪阳性染色结果呈橘红至红色，具体颜色因脂质种类和浓度而定。

操作步骤：*（仅供参考）*

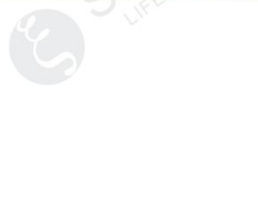
1. 取材后使用 2-8℃冰箱预冷的 G2161-中性福尔马林固定液或 P1110-4%组织细胞固定液充分固定后切片或液氮速冻取材制备新鲜冰冻切片，通常建议 10-12um。
2. 切片恢复室温后，滴加试剂(A):油红 O 染色缓冲液覆盖切片处理 2-5min。（见注意事项 1）
3. 倾去多余缓冲液无需清洗，直接滴加预热好的试剂(B):油红 O 染色液覆盖切片后室温染色 15min。
4. 倾去多余染色液，滴加等量试剂(C):油红 O 染色分化液分化处理 2-5min 至切片颜色均匀。
5. 蒸馏水浸洗 1min 充分去除分化液，滴加试剂(D): Mayer 苏木素染色液染色 2min。
6. 蒸馏水洗 1min 后自来水反蓝 5-10min 至细胞核呈清晰蓝色。
7. 使用预热融化的 S2150-甘油明胶封片剂封片后光学显微镜观察。

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. 脂质易溶于有机溶剂，一般使用冰冻切片进行染色，推荐使用预冷的 G2161-中性福尔马林固定液或 P1110-4%组织细胞固定液进行组织固定后制备切片，同时不建议对速冻组织切片使用丙酮进行二次固定。
2. 试剂(A):油红 O 染色缓冲液具备固定和缓冲作用，用于速冻切片可适当延长孵育时间（5min）对组织进行适当固定。如组织为固定后切片可适当缩短孵育时间（2min）。
3. 染色液和缓冲液较粘稠，推荐吸头剪口后吸取，或冬季使用前适当预热（25-28℃）。同时因为较粘稠，无法完全倾倒干净属正常现象，直接遵循操作步骤进行下一步即可。
4. 染色结果不能长期保存，应尽快观察及照相。
5. 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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V02

Modified Oil Red O Stain Kit, Environmental Friendly

Cat: G1263
Size: 4×50mL
Storage: RT, avoid light, valid for 1 year.

Kit Components

Reagent	4×50mL	Storage
Reagent(A):Oil Red O Buffer	50mL	RT
Reagent(B):Oil Red O Staining Solution	50mL	RT, avoid light
Reagent(C):Oil Red O Differentiation Solution	50mL	RT
Reagent(D): Mayer Hematoxylin Solution	50mL	RT, avoid light

Introduction

Lipid is the general name of neutral fat, lipids and their derivatives. Its common physical characteristics are insoluble in water and soluble in organic solvents (such as ethanol, acetone, etc.). Sudan II, Sudan III, Sudan IV, Sudan black B, oil red O and other methods are often used for staining of lipids in tissues. The traditional method uses sudan dyes. Recently, it has been found that azo dye oil red O is more suitable for fat dyeing. Oil red O is a strong fat dye, which is easy to combine with neutral fat and has a slightly poor binding force with phospholipids. Its dyeing principle is generally considered that fat is dyed by similar miscibility or physical adsorption. The solubility of dyes in intracellular lipids is greater than that in original solvents. Therefore, when dyeing, dyes are transferred from organic solvents to lipids to dye fats.

Modified Oil Red O Stain Kit(Polyol Method) uses an odorless and low volatile environment-friendly solvent to replace classical solvent with certain risks, which greatly reduces the use risk and operation difficulty without affecting the location and size of lipids, and is very friendly to experimental operators. The result of fat positive staining is orange red to red, and the specific color depends on the type and concentration of lipids.

Protocol(*for reference only*)

1. After sampling, fix tissues with G2161-Neutral buffered Formalin,10% or P1110-Paraformaldehyde, 4% precooled by 2-8 ℃ refrigerator for full fixation or quick freezing with liquid nitrogen to prepare fresh frozen sections. Generally, the section is recommended to be cut in 10-12um.
2. Before staining, rewarm the slices to room temperature. Drop the Oil Red O Buffer onto the section for 2-5min. (see Note 1)
3. Pour out the excess buffer without washing, and directly add the preheated Oil Red O Staining Solution to cover the section and dye at room temperature for 15min.
4. Pour out the excess staining solution and add the same amount of Oil Red O Differentiation Solution for differentiation treatment for 2-5min until the section color is uniform.
5. Soak in distilled water for 1min to fully remove the differentiation solution, and stain with Mayer Hematoxylin Solution for 2min.
6. After washing with distilled water for 1min, blue with tap water for 5-10min until the nucleus is clear blue.
7. Use the preheated and melted S2150-Glycerol Gelatin aqueous slide mounting medium to seal the section and observe it under the optical microscope.

Result

Neutral Fat	Orange or Red
Phospholipid	Pink Red
Nucleus	Blue

Note

1. Lipids are easily soluble in organic solvents. Generally, frozen sections are used for staining. It is recommended to use precooled G2161-Neutral buffered Formalin,10% or P1110-Paraformaldehyde,4% for fixation to prepare frozen sections. At the same time, it is not recommended to use acetone for secondary fixation of quick-frozen tissue sections.
2. Reagent(A):Oil Red O Buffer has the functions of fixation and buffering. For quick-frozen sections, the incubation time (5min) can be appropriately prolonged to properly fix the tissues. If the tissue is sectioned after fixation, the incubation time can be appropriately shortened (2min).

3. The dye solution and buffer solution are viscous. It is recommended to suck them after cutting the mouth of the suction head, or preheat them properly before use in winter (25-28 ℃). At the same time, it is normal that it cannot be completely dumped because it is viscous. You can directly follow the operating steps for the next step.
4. The staining results cannot be stored for a long time, so they should be observed and photographed as soon as possible.
5. For your safety and health, please wear lab clothes and disposable gloves.

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