V02

脂质荧光染色试剂盒（尼罗红法）

货号：G1264
规格：2×50mL
保存：-20℃，避光保存，有效期 1 年。

产品组成：

名称		2×50mL	保存
试剂（A）：脂质固定液		50mL	2-8℃，避光
试剂（B）：染色工作液	B1：染色储备液	0.2mL	-20℃，避光
	B2：缓冲稀释液	40mL	2-8℃
临用前，按 B1:B2=1:200 的比例配制染色工作液，注意避光操作，在 1h 内使用完毕。			

产品介绍：

脂质是一种低极性（疏水性）的天然大分子混合物，包括脂肪酸及其衍生甘油酯和磷脂，以及固醇类物质，例如胆固醇。细胞浆脂滴的形成是正常细胞生理过程，脂滴由中性脂质构成，通常为甘油三酯脂滴，作为能量储备；少部分为胆固醇酯脂滴，作为过多细胞胆固醇的存储。尼罗红(Nile red)染色剂是一种亲脂性的恶嗪类黄光染料。与腊酯、甘油三酯以及各种脂肪酸等脂类物质结合后，在激发波长 543nm 的激发下，显示强烈橘黄色荧光(散发波长 598m)。同时在紫外光的照射下显示红色。

脂质荧光染色试剂盒（尼罗红法）优点在于提供复合荧光染色液，可同时对脂类物质和细胞核进行组织原位染色。在绿光激发下，脂滴呈橘黄色，在紫外光激发下，细胞核呈蓝色，适用于冰冻切片和细胞样本。本产品为即用型试剂盒，性能稳定，着色清晰灵敏。

自备材料：

1×PBS、1.5 毫升离心管、荧光显微镜

操作步骤：(*仅供参考*)

临用前取出试剂(B1)和试剂（B2）放置恢复至室温，按 B1:B2=1:200 的比例配制染色工作液，避免光照，然后进行下列操作。

一、冰冻切片染色

1. 取出待测的冰冻切片，复温 3min。
2. 向切片上滴加试剂(A):固定液，铺满整个组织，室温固定 15-20min。1×PBS 洗 3 次，每次 1min。
3. 向切片上滴加染色工作液，室温避光孵育 10min。
4. 1×PBS 清洗切片 1-2 次，每次 30s，至无红色染料脱出。
5. 加上盖玻片制备临时切片或使用 S2100-抗荧光衰减封片剂封片，然后在荧光显微镜下观察：激发波长 543nnm，散发波长 598nm（脂滴着色）和紫外光（核着色）。

二、贴壁细胞染色

1. 小心吸除培养皿或六孔板里的培养液。
2. 小心加入 1ml 1×PBS，温柔清洗细胞表面，重复 1-2 次每次 30s。
3. 小心吸除培养皿或六孔板里的清洗液。
4. 加入 1ml 试剂(A):固定液，覆盖细胞表面，固定 10-15min。小心吸除固定液。
5. 加入 1ml 1×PBS，清洗细胞表面，重复 1-2 次。小心吸除清洗液。
6. 加入适量染色工作液，室温避光孵育 10min，小心吸除染色工作液。
7. 小心加入 1ml 1×PBS，摇晃清洗细胞表面 30s-1min，重复 1-2 次。
8. 最后一次清洗液不弃去，注意避光保存并在 30min 内用荧光显微镜下观察：激发波长 543nnm，散发波长 598nm（脂滴着色）和紫外光（核着色）。

染色结果：

脂类物质	红色或橘黄色荧光

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细胞核	亮蓝色荧光

注意事项：

1. 染色工作液现用现配，不宜提前配置。
2. 根据组织和细胞的差异，可以适当调整染料稀释比例（1：100-1：400），以增强或降低荧光强度。
3. 细胞培养液里避免使用脂类物质。染色孵育和观察时，避免强光照射，以免荧光淬灭。
4. 使用时，试剂(B1)：染色液避免反复冻融，可分装成小规格进行保存。
5. 建议染色完成后，即刻进行荧光检测分析，如没有荧光显微镜，可以选择紫外下观察。
6. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

相关产品：

P1110　4%组织细胞固定液
G1261　改良油红O染色试剂盒
G1263　改良油红O染色试剂盒（环保型）
G1511　苏丹III染色液（试剂盒）

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Lipid Fluorescent Staining Kit (Nile Red Method)

Cat: G1264
Size: 2×50mL
Storage: -20℃, avoid light, valid for 1 year.

Kit Components

Reagent		2×50mL	Storage
Reagent(A): Lipid Fixative		50mL	2-8℃， avoid light
Reagent(B): Stain Working Solution	B1:Dyeing Stock Solution	0.2mL	-20℃，avoid light
	B2:Buffer Diluent	40mL	2-8℃
Before the use, prepare the dyeing working liquid according to the ratio of B1: B2=1:200, pay attention to the light avoidance operation, and finish using it within 1h.			

Introduction

Lipids are a natural mixture of low polar (hydrophobic) natural macromolecules, including fatty acids and their derived glycerides and phospholipids, as well as steroids, such as cholesterol. The formation of cytoplasmic lipid droplets is a normal cell physiological process, consisting of neutral lipids, usually triglyceride lipid droplets, as energy reserves; a few are cholesterol ester lipid droplets, as the storage of excessive cellular cholesterol. Nile red (Nile Red) stain is a lipophilic oxazine yellow light dye. In combination with lipids such as wax ester, triglycerides and various fatty acids, a strong orange fluorescence (emission wavelength of 598m) was displayed at an excitation wavelength of 543nm. Also shows red under irradiation with UV light.

The advantage of the Lipid Fluorescent Staining Kit (Nile Red Method) is to provide a composite fluorescent staining solution for simultaneous tissue in situ staining of lipids and nuclei. Under green light excitation, the orange-colored lipid droplets, and the blue cell nuclei upon UV excitation, were suitable for frozen sections and cell samples. This product is ready to use kit, stable performance, clear and sensitive coloring.

Self Provided Materials

1× PBS, 1.5 ml Centrifuge tube, Fluorescence microscope

Protocols(*for reference only*)

Before use, take out the reagent (B1) and reagent (B2) and put them back to room temperature. Prepare the dyeing working solution according to the ratio of B1: B2 = 1:200, avoid the light, and then carry out the following operations.

For Frozen Section Stain

1. Take out the prepared frozen section, place it for 3min, and rewarm it to room temperature.
2. Add the Reagent(A): Lipid Fixative to the section, cover the whole tissue and fix at room temperature for 15-20min. Wash with 1×PBS 3 times, 1min for each.
3. Add the Stain Working Solution to the sections and incubate for 10min at RT avoide light.
4. Wash the section 1-2 times with 1×PBS for 30s each til no red pigment comes out.
5. Cover slides were added to prepare temporary sections or use S2100-antifluorescence decaying tablets, and then observed under a fluorescence microscope: excitation wavelength 543nnm, emission wavelength 598nm (lipid droplet staining) and ultraviolet light (nuclear staining).

For Adherent Cells Stain

1. Remove the medium from the dish or six-well plate carefully.
2. Add 1ml 1×PBS carefully, clean the cell surface gently and repeated 1-2 times, each for 30s.
3. Remove the cleaning solution from the culture dish or from the six-well plate carefully.
4. Add 1ml Reagent (A): Fixation Solution, cover the cell surface and fix for 10-15min. Remove the fixative fluid carefully.
5. Add 1ml 1×PBS and clean the cell surface, repeated 1-2 times. Remove the washing liquid carefully.
6. Add appropriate amount of staining solution and incubated for 10min at room temperature in the dark.
7. Add 1ml 1×PBS carefully, and wash the cell surface for 30s-1min by shaking, repeat 1-2 times.
8. Cleaning liquid in the last step is not abandoned, pay attention to avoid light and observe by fluorescence microscope within 30 min: excitation wavelength 543nnm, emission wavelength 598nm (lipid droplet coloring) and ultraviolet light (nuclear staining).

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Result

Lipid substances	Red or orange-yellow fluorescence
Nucleus	Bright blue fluorescence

Note

1. The Stain Working Solution should be configured before the use , not in advance.
2. Depending on the tissue and cell differences, the dye dilution ratio (1:100-1:400) can be adjusted appropriately to enhance or decrease the fluorescence intensity.
3. Avoid using lipids in the cell culture medium. During staining, incubation and observation, avoid intense light irradiation and avoid fluorescence quenching.
4. When you use this kit, the reagent (B1): Dyeing Stock Solution should be avoid repeated freezing and thawing, can be divided into small specifications for preservation .
5. It is recommended that fluorescence detection and analysis be performed immediately after the staining is completed. If there is no fluorescence microscope, UV observation can be selected.
6. For your safety and health, please wear lab suits and wear disposable gloves.