线粒体活性染色试剂盒

货号：G1573
规格：3×10mL
保存： -20℃，避光保存，有效期 6 个月。

产品组成：

名称	3×10mL	保存
试剂(A): 保存液	10mL	-20℃
试剂(B): 染色液	10mL	-20℃, 避光
试剂(C): 清理液	10mL	-20℃

产品介绍：

线粒体是细胞中重要的细胞器，其主要功能是提供细胞内各种物质代谢所需要的能量。在光学显微镜下线粒体呈现为颗粒状、棒状或弯曲细线。詹纳斯绿 B（Janus green B），是一种毒性较小的碱性染料。它可以对活细胞进行直接染色，在细胞质内可以看到被染成蓝绿色的线状或颗粒小体的线粒体。线粒体所以能显示出蓝绿色，是由于线粒体中具有细胞色素氧化酶系统，它使染料始终处于氧化状态呈蓝绿色，而在周围的细胞质中的染料被还原呈无色。

线粒体活性染色试剂盒其适用于各种线粒体（动物、人体、植物、昆虫等）制备物的功能检测。产品严格无菌，即到即用，活体检测，操作简捷，性能稳定。

自备材料：

EP 离心管、光学显微镜

操作步骤：(*仅供参考*)

实验开始前，将染色液提前从冰箱取出置于 4℃里融化，并放在暗室里。然后进行下列操作。

一、纯化线粒体染色

1. 从纯化的线粒体样品中移出 5 至 100 ul（含 10^6 细胞中提取的线粒体）到新的预冷 1.5 ml 离心管，置于冰槽里（注意：线粒体须均匀分布，没有聚集成团）。
2. 加入等量微升的染色液，轻柔混匀，放进暗室里，在室温下孵育 1 分钟。
3. 即刻移取 10 ul 到载玻片上，放上盖玻片。
4. 在光学显微镜油镜下进行观察：功能完整的线粒体呈现蓝绿色圆形或椭圆形颗粒。蓝绿色强度显著减弱或呈现无色，表明线粒体细胞色素氧化酶系统功能不全或功能丧失。

二、活体细胞染色

1. 将待测细胞（1×10^6 细胞）移入到 1.5 ml 离心管。
2. 放进微型台式离心机离心 1 分钟，转速为 500g，小心抽去上清液。
3. 加入 500ul 清理液，加入 500ul 染色液，充分混匀。
4. 放进暗室里，在冰槽里孵育 20 分钟，即刻移取 10ul 到载玻片上，放上盖玻片。
5. 在光学显微镜油镜下进行观察：功能完整的线粒体呈现蓝绿色线状或颗粒小体。

注意事项：

1. 建议操作在无菌状态下进行，线粒体样品操作需在低温下进行，建议快速操作。
2. 操作时，需戴手套，染色完成后，即刻进行显微镜观察分析。

Mitochondrial Activity Detection Kit

Cat: G1573
Size: 3×10mL
Storage: -20℃, avoid light, valid for 6 months.

Kit Components

Reagent	3×10mL	Storage
Reagent(A): Preservation solution	10mL	-20℃
Reagent(B): Staining Solution	10mL	-20℃, avoid light
Reagent(C): Cleaning Solution	10mL	-20℃

Introduction

Mitochondria is an important organelle in cells, which of main function is to provide energy for metabolism of various substances in cells. Janus Green B is an alkaline dye with less toxicity. It can stain the living cells directly, and in the cytoplasm, we can see the mitochondria stained with blue-green linear or granular bodies. The reason why mitochondria can show blue-green is that there is cytochrome oxidase system in mitochondria, which makes the dye always in the oxidation state blue-green, while the dye in the background is reduced to colorless.

Mitochondrial Activity Detection Kit is suitable for the functional detection of various mitochondrial (animal, human, plant, insect, etc.) preparations. The product is strictly sterile, ready to use, in vivo detection, simple operation and stable performance.

Self Provided Materials

EP tube, Optical microscope

Protocol (*for reference only*)

Before the experiment, remove the reagent out to melt in the darkroom. Then do the following steps.

Purified Mitochondrial Staining

1. Remove 5 to 100 ul (including mitochondria extracted from 10^6 cells) from the purified mitochondria sample to a new precooling 1.5 ml EP tube, and place it in an ice bath (*Note: mitochondria must be evenly distributed and not clustered*).
2. Add the same amount staining solution and mix gently.Incubate it in the ice bath avoid light at RT for 1 min.
3. Immediately remove 10 ul onto the slide and cover.
4. View under the oil immersion: the active mitochondria present blue-green round or oval particles.
5. The blue-green intensity is significantly weakened or colorless, indicating that the mitochondrial cytochrome oxidase system is not fully functional or lost.

Living cell staining

1. Transfer the cells (1×10^6 cells) to 1.5 ml EP tube.
2. Centrifugal for 1 minute with the rotating speed of 500g, and carefully remove the supernatant.
3. Add 500 ul Cleaning Solution into the tube.Add 500 ul Staining Solution into the tube and mix well.
4. Incubate it in the ice bath and avoid light for 20 min.Immediately remove 10 ul onto the slide and cover.
6. View under the oil immersion lens:the active mitochondria present blue-green round or oval particles.

Note

1. It is recommended to operate in sterile state.Wear gloves during operation.
2. The operation of mitochondrial samples should be carried out fast at low temperature.
3. It is suggested to view and analysis immediately after dyeing.