V02

细胞自噬染色检测试剂盒(MDC 法)

货号：G0170
规格：100T
保存：-20℃，避光保存，有效期1年。

产品组成：

名称	100T	保存
试剂(A): MDC染色液	1mL	-20℃,避光
试剂(B): 10×清洗液	20mL	2-8℃
试剂(C): 缓冲收集液	10mL	2-8℃

产品介绍：

自噬(autophagy)是细胞受到刺激后吞噬自身的细胞质或细胞器，最终将吞噬物在溶酶体内降解的过程,自噬体(autophagosome)为双层膜包被的圆形或椭圆形结构，内含细胞质、长寿蛋白质和异常蛋白聚集物,损伤或多余细胞器如线粒体、粗面内质网和微体、病毒和细菌等。

单丹磺酰尸胺(Dansylcadaverine，MDC)是一种荧光色素，是嗜酸性染色剂，通常被用于检测自噬体形成的特异性标记染色剂，其检测激发滤光片波长 355nm。阻断滤光片波长 512nm。细胞自噬染色检测试剂盒(MDC 法)，适用于培养细胞的自噬染色，又称为 MDC 染色液，可与 EB 合用双染。

自备材料：

荧光显微镜、低速离心机、EB染色液、载玻片、盖玻片

操作步骤：(*仅供参考*)

(一)悬浮细胞染色

1. 10×清洗液用去离子水稀释至1×。
2. 800g离心5min，收集细胞，用300～400μl的1×清洗液清洗细胞1次，弃上清。
3. 加入适量的1×清洗液重悬细胞，计数并调节细胞浓度至10^6/mL。
4. 取90μl的细胞悬液至新的EP管中，加入10μl的MDC染色液，轻轻混匀。室温避光染色15～30min。（见注意事项1）
5. 800g离心5min，收集细胞，用300～400μl的1×清洗液清洗细胞2次，弃上清。
6. 加入100μl的缓冲收集液重悬细胞，滴加于载玻片上并加盖玻片。
7. 荧光显微镜下观察(阻断滤光片波长512nm)，计数并拍照。

(二)贴壁细胞染色

1. 10×清洗液用去离子水稀释至1×清洗液，去除培养基并用1×清洗液清洗细胞。
2. 用1×清洗液将适量MDC染色液稀释10倍，然后加到孔板里室温避光孵育15～30min。（见注意事项2）
3. 染色后用1×清洗液清洗2次，每次3min。
4. 倒置荧光显微镜下观察(阻断滤光片波长512nm)，计数并拍照。

染色结果：

正常细胞	细胞被均匀染成黄绿色荧光
凋亡细胞	染色质浓缩，细胞核碎裂成点状，被染成大小不一、致密浓染的绿色颗粒

注意事项：

1. MDC染色液和EB试剂合用可区分出正常细胞、凋亡细胞及坏死细胞。有一定毒性，请小心操作。如需联合染色可在加入MDC染色液的细胞悬液中加入终浓度为0.2μM的EB染色液，后续正常操作。
2. 这里可按照孔板添加培养基体积减半的原则加入10倍稀释的MDC染色液，也可根据细胞数酌情调整稀释比，避免浓度过高产生假阳性。
3. 操作过程中应注意减少试剂暴露于强光下的时间。
4. 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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Autophagy/Cytotoxicity Dual Stain Kit(MDC Method)

Cat: G0170
Size: 100T
Storage: -20℃,avoid light,valid for 1 year.

Kit Components

Reagent	100T	Storage
Reagent A : MDC Stain Solution	1mL	-20℃,avoid light
Reagent B : 10×Wash buffer	20mL	2-8℃
Reagent C : Collection buffer	10mL	2-8℃

Introduction

Autophagy is a process that cells are stimulated to phagocytize their own cytoplasm or organelles and eventually degrade the phagocytes in lysosomes.Autophagosome is a circular or elliptic structure encapsulated by bilayer membranes. It contains cytoplasm,longevity proteins,complex of abnormal proteins,damages or redundant organelles,viruses and bacteria.

Dansylcadaverine (MDC) is a fluorescent pigment and an eosinophilic stain. It is usually used to detect the formation of autophages as a specific marker dye. Its detection excitation filter wavelength is 355 nm and the blocking filter wavelength is 512 nm. The Autophagy/Cytotoxicity Dual Stain Kit(MDC Method) is suitable for autophagy staining of cultured cells and can counterstain with EB.

Self Provided Materials

Fluorescence microscope,Low-speed centrifuge,EB stain

Protocol(*for reference only*)

For Suspension Cell Stain

1. Dilute 10× Wash buffer to 1× with distilled water.
2. 800g centrifugation for 5 min.Collect the cell in the tube and wash once with 1×Wash buffer of 300-400 μl. Remove the supernatant.
3. Add a certain amount of 1 ×Wash buffer to suspend cells,count and regulate the cell concentration to 10^6/mL.
4. Take 90μL cell suspension into a new EP tube and add 10 μL MDC stain solution and gently mix well.Incubate for 15-45 min at room temperature and avoid light. (See Note 1)
5. 800g centrifugation for 5 min.Collect the cell in the tube and wash twice with 1×Wash buffer of 300-400 μl. Remove the supernatant.
6. Add 100μl Collection buffer to suspend the cell,drop it onto the slide and cover the slide.
7. View under the fluorescence microscope (blocking filter wavelength is 512 nm),count and photograph.

For Adherent Cell Stain

1. remove the culture medium and wash with Diluted 1× Wash buffer.
2. Dilute MDC Stain Solution with 1× Wash buffer by 1:10,then add to cultivation plate and stain for 15-45min.
3. Wash with 1 ×Wash buffer twice and 3min for each time.
4. View under the fluorescence microscope (blocking filter wavelength is 512nm),count and photograph.

Result

Normal cells	Cells are evenly dyed yellow-green fluorescence.
Apoptotic cells	Collapsed chromatin and punctate nucleus are dyed into dense green particles of varying sizes.

Note

1. The combination of MDC stain solution and EB reagent can distinguish normal cells, apoptotic cells, and necrotic cells. It has certain toxicity, please handle with caution. If combined staining is required, a final concentration of 0.2 can be added to the cell suspension with MDC staining solution μ M's EB staining solution, followed by normal operation.
2. Here, 10 times diluted MDC staining solution can be added according to the principle of reducing the volume of the culture medium added to the well plate by half. The dilution ratio can also be adjusted according to the number of cells to avoid false positives caused by high concentration.
3. During the operation,pay attention to reducing the exposure time of reagents to strong light.
4. For your safety and health,please wear experimental clothes and disposable gloves.