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 Stain	1mL	-20℃，避光
试剂(B): 10×Wash buffer	20mL	2-8℃
试剂(C): Collection buffer	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、载玻片、盖玻片

操作步骤：(*仅供参考*)

(一)、MDC单独染色：

1. 用去离子水稀释10×Wash buffer至1×。
2. 800g离心5min，收集细胞，用300～400μl的1×Wash buffer清洗细胞1次，弃上清。
3. 加入适量的1×Wash buffer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 Stain，轻轻混匀。室温避光染色15～45min。
5. 800g离心5min，收集细胞，用300～400μl的1×Wash buffer清洗细胞2次，弃上清。
6. 加入100μl的Collection buffer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(激发滤光片波长355nm，阻断滤光片波长512nm)，计数并拍照。

注:*贴壁细胞可以不消化，直接去除培养液，用1×Wash buffer清洗后进行染色。MDC的量根据细胞数量进行调整。染色时间可适当延长根据染色结果进行调整，染色结束后，用1×Wash buffer清洗观察即可，不加Collection buffer。*

(二)、与EB双染色：

1. 用去离子水稀释10×Wash buffer至1×。
2. 800g离心5min，收集细胞，用300～400μl的1×Wash buffer清洗细胞1次，弃上清。
3. 加入适量的1×Wash buffer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，分别加入10μl的MDC Stain和0.2μM EB染色液，轻轻混匀。
5. 滴加于在玻片上，室温避光染色15-30min，加盖玻片。
6. 荧光显微镜下观察(激发滤光片波长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 染色和EB试剂有一定毒性，请小心操作。
2. 吖啶橙染色常与EB染色合用，可区分出正常细胞、凋亡细胞及坏死细胞。

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。

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	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*)

（一）MDC staining alone

1. Dilute 10× Wash buffer to 1× with distilled water.
2. 800g centrifugation for 5 minutes.Collect the cell in the tube and wash once with 1×Wash buffer of 300-400 μl. Remove the supernatant.
3. Add appropriate amount of 1 ×Wash buffer to suspend cells, count and regulate the cell concentration to 10^6/mL.
4. Incubate for 15-45 minutes at room temperature and avoid light.
5. 800g centrifugation for 5 minutes.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 (excitation filter wavelength is 355 nm, blocking filter wavelength is 512 nm) , count and photograph.

Note:For adherent cells, can remove the culture medium directly without digestion and stain after washing with 1 × Wash buffer.Adjust the amount of MDC according to the cell number. The dyeing time can be appropriately prolonged and adjusted according to the dyeing results. After dyeing, wash with 1× Wash buffer and view without adding Collection buffer.

（二）Counterstain with EB

1. Dilute 10× Wash buffer to 1× with distilled water.
2. 800g centrifugation for 5 minutes.Collect the cell in the tube and wash once with 1×Wash buffer of 300-400 μl. Remove the supernatant.
3. Add same equal of 1 ×Wash buffer to suspend cells, count and regulate the cell concentration to 10^6/mL.
4. Take 90 μl cell suspension into the new tube, add 10 μl MDC Stain and 0.2μM EB stain and gently mix.
5. Drop onto the slide and stain it at room temperature for 15-30 minutes without light. Cover the slide.
6. View under the fluorescence microscope (excitation 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. MDC Stain and EB Reagent have certain toxicity. Please pay attention to the protection during operation.
2. This kit is often used with acridine orange to distinguish normal cells, apoptotic cells and necrotic cells.
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.