Autophagy/Cytotoxicity Dual Staining Kit (MDC/PI)

Introduction

Autophagy is a highly conserved degradation process within cells, which essentially provides energy for cell survival by degrading excess or abnormal components through the lysosomal system under nutrient deficiencies, environmental stress, or other stressful conditions. Abnormalities in the autophagy process have been implicated in a variety of disease states, including neurodegenerative diseases, cancer, metabolic disorders, and infections, among others. Autophagy studies can help uncover how cells adapt to and respond to environmental stresses and maintain homeostasis through self-degradation, providing direction for the development of new therapeutic strategies.

Autophagy/Cytotoxicity Dual Staining Kit (MDC/PI) provides two fluorescent probes, monodansylcadaverine (MDC) and propidium iodide (PI). MDC is currently the most commonly used fluorescent probe for the detection of autophagy, which can specifically bind membrane lipids to effectively label autophagosomes. Because MDC is an eosinophilic probe, some acidic membrane structures can also be labeled, so normal cells will also have a certain fluorescence background. PI can be used to label cells in advanced apoptotic or necrotic stages. Combining these two probes allows for simultaneous detection of autophagy and toxicity.

This kit can be used for the detection of cultured cells, not cryopreserved cells, fixed cells, fixed tissues, or tissue sections.

Composition and Storage

Components \ Size	500 Tests	2000 Tests	Storage
MDC (1000×)	50 μL	200 μL	-20°C away from light
PI (500×)	100 μL	400 μL	-20°C away from light
Assay Buffer (10×)	30 mL	120 mL	-20°C
Shipping: Blue ice	Shelf life: 1 year		

Protocol

1. Preparation before the experiment

1) According to the experimental needs, dilute an appropriate amount of Assay Buffer (10×) with ddH_2O to make Assay Buffer (1×).

2) Dilute both MDC (1000×) and PI (500×) with Assay Buffer (1×) to make MDC/PI (1×) according to experimental needs. For example, dilute 1 μL of MDC (1000×) and 2 μL of PI (500×) in 997 μL of Assay Buffer (1×) to make 1 mL of MDC/PI (1×).

***Note:** Care should be taken to protect MDC and PI from light when using.

2. For adherent cell (96-well plate)

1) For 96-well plates, seed 5×10^4 cells per well with a seeding volume of 100 μL per well. Incubate cells overnight to allow them to be adherent.

2) Treat experimental group cells with interested compound according to the experimental design, while an untreated control group is prepared. If a positive control is required, wash cells once with PBS, then add EBSS, and incubate in a 37°C incubator for 1-4 h to induce autophagy.

***Note:**

a) After the cells are treated to induce autophagy, they are easily detached and need to be gentle in subsequent operations. For cells with weak adherent capacity, it is recommended to use pre-coated plates such as polylysine or gelatin before performing experiments.

b) If you need EBSS, you can purchase Earle's Balanced Salt Solution (EBSS) (Cat. No. K2719). For general cells, EBSS treatment for 1-2 h is sufficient.

3) After treatment, discard the medium and wash the cells 1-2 times with PBS. For 96-well plates, add 100 μL of MDC/PI (1×) per well and incubate in a 37°C incubator protected from light for 15 min.

***Note:** The incubation time can be adjusted according to the specific experiment, but is generally within 10-60 min.

4) After incubation, discard the MDC/PI staining solution and wash the cells 1-3 times with Assay Buffer (1×).

5) Discard Assay Buffer (1×) and add 100 μL of Assay Buffer (1×).

6) The result can be detected by a fluorescence microscope or a fluorescence microplate reader. The recommended detection wavelength for MDC is Ex/Em=335/512 nm. The recommended detection wavelength for PI is Ex/Em=535/615 nm.

3. For suspension cell

1) Treat experimental group cells with interested compound according to the experimental design, while an untreated control group is prepared. If a positive control is required, wash cells once with PBS, then add EBSS, and incubate in a 37°C incubator for 1-4 h to induce autophagy.

***Note:** Earle's Balanced Salt Solution (EBSS) (Cat. No. K2719) is available for purchase if EBSS is required. For general cells, EBSS treatment for 1-2 h is sufficient.

2) After treatment, centrifuge at room temperature at 1000 rpm for 5 min. Discard the medium and wash the cells 1-2 times with PBS. For $0.5\text{-}1\times10^5$ cells, gently resuspend the cells with 100 μL of MDC/PI (1×) and incubate in a 37°C incubator for 15 min in the dark.

*Note: The incubation time can be adjusted according to the specific experiment, but is generally within 10-60 min.

3) After incubation, centrifuge at room temperature at 1000 rpm for 5 min. Discard the MDC/PI staining solution and wash cells 1-3 times with Assay Buffer (1×).

4) Centrifuge to discard the Assay Buffer (1×) and add an appropriate amount of Assay Buffer (1×) to gently resuspend the cells.

7) The recommended detection wavelength for MDC is Ex/Em=335/512 nm. The recommended detection wavelength for PI is Ex/Em=535/615 nm. A portion of the cell suspension can be prepared into smears for fluorescence microscopy. Assays can also be performed directly by flow cytometry.

Note

1. MDC and PI are sensitive to light, so protect them from light.
2. PI is a carcinogen, please pay attention to protection when using.
3. For your safety and health, please wear lab coats and gloves during the experiment.
4. For research use only. Not to be used in clinical diagnostic or clinical trials.

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