Autophagy Staining and Detection Kit (MDC Method)

C1511655

Storage: -20°C. Room Temperature. Store in the dark.
Shipping: Shipped with ice packs.

Introduction:

Autophagy is a process in which cells engulf their own cytoplasm or organelles in response to stimulation, and ultimately degrade the engulfed materials within lysosomes. Autophagosomes are spherical or elliptical structures surrounded by a double membrane, containing cytoplasm, long-lived proteins and aggregates of abnormal proteins, damaged or redundant organelles such as mitochondria, rough endoplasmic reticulum and microbodies, as well as viruses and bacteria. Autophagy is an evolutionarily conserved degradation process that degrades long-lived proteins, organelles, and other cytoplasmic components via the lysosomal pathway. Activation of the autophagic pathway is essential for diverse cellular functions, including survival under starvation, clearance of intracellular components, development, and immune responses. Dansylcadaverine (MDC) is an acid-responsive fluorescent dye commonly used as a specific marker for detecting autophagosome formation. Its excitation filter wavelength is 355–380 nm, and its barrier filter wavelength is 512–530 nm. The Cellular Autophagy Staining Detection Kit (MDC Method), also known as MDC Staining Solution, is suitable for fluorescent staining and observation of autophagy in cultured cells. It can also be used to detect the proportion of autophagic cells by FACS, and can be used for double staining in combination with EB. This reagent is for research use only and is not intended for clinical diagnosis or other applications.

Product Components and Storage Conditions:

C1511655	Component	100T	Storage
C1511655A	MDC Stain(10×)	1mL	-20℃. Store in the dark.
C1511655B	Stain Buffer	50mL	RT
C1511655C	Wash Buffer	100mL	RT

Materials Required:

1. Low-speed centrifuge, 1.5 mL centrifuge tubes, glass slides, cover slips, 96-well plates, fluorescence microscope, flow cytometer.
2. Cell culture medium, distilled water, phosphate-buffered saline (PBS).

Protocol (For Reference Only):

1 Slide Method:

(1) Collect cells. Wash cells once with 300–500 μL Wash Buffer, centrifuge at 800–1000 rpm for 5 min, and discard the supernatant. Resuspend cells in an appropriate volume of Stain Buffer, count cells, and adjust the concentration to 1×10^6 cells/mL.
(2) Transfer 90 μL of cell suspension to a new 1.5 mL centrifuge tube, add 10 μL MDC Stain (10×), and mix gently.
(3) Stain at 37 °C or room temperature in the dark for 15–60 min.
(4) Centrifuge at 800–1000 rpm for 5 min, discard the supernatant, and collect cells. Wash cells twice with 300–500 μL Wash Buffer, centrifuge at 800–1000 rpm for 5 min, and discard the supernatant.
(5) Resuspend cells in 100 μL Wash Buffer, mount on a glass slide, and cover with a cover slip.
(6) Observe, count, and capture images under a fluorescence microscope. The percentage of MDC-positive cells in the drug-treated group can also be detected by flow cytometry.

2 96-Well Plate Method:

(1) Gently remove the culture medium from the 96-well plate. Add 10 μL MDC Stain (10×) and 90 μL Stain Buffer to each well. Incubate at 37 °C in 5% CO_2 in the dark for 15–60 min.
(2) Add 100 μL Wash Buffer to each well and wash 2–3 times. Detect and observe with a fluorescence microscope or flow cytometer.

3 MDC & EB Double Staining:

(1) Collect cells. Wash cells once with 300–500 μL Wash Buffer, centrifuge at 800–1000 rpm for 5 min, and discard the supernatant. Resuspend cells in an appropriate volume of Stain Buffer, count cells, and adjust the concentration to 1×10^6 cells/mL.
(2) Transfer 90 μL of cell suspension to a new 1.5 mL centrifuge tube, add 10 μL MDC Stain (10×) and 0.2 μM EB Stain, mix gently. Mount on a glass slide, stain at room temperature in the dark for 15–60 min, and cover with a cover slip.
(3) Detect and observe with a fluorescence microscope or flow cytometer.

Staining Results:

1. Fluorescence microscopy: The drug-treated group showed stronger fluorescence intensity and more bright green MDC-labeled puncta.
2. FACS analysis: The percentage of MDC-positive cells in the drug-treated group was higher than that in the control group.

Precautions:

1. MDC Stain and EB are harmful to humans; handle with care.
2. Before cell collection, cells can be cultured with different concentrations of drugs for a certain period to induce autophagy.
3. Minimize exposure of reagents to strong light during operation.
4. Wash Buffer can be replaced with PBS.
5. Use the reagents promptly after opening to avoid affecting experimental results.
6. For your safety and health, wear a lab coat and disposable gloves during operation.