Autophagy/Cytotoxicity Dual Staining Kit

Item No. 600140

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit. After opening, remove the components and stored as stated below.

Item Number	Item	100 Tests Quantity/Size	Storage
600141	Cell-Based Monodansylcadaverine	1 vial/100 µl	-20°C
10009322	Cell-Based Assay Buffer Tablet	1 vial/5 tablets	RT
10011234	Cell-Based Propidium Iodide Solution	1 vial/250 µl	4°C
10011018	Cell-Based Tamoxifen (100 mM)	1 vial/50 µl	-20°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

E-Mail: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section, on page 3, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Microscopy: a fluorescence microscope with filter sets for detection of DAPI and rhodamine or Texas Red
2. Plate Reader Detection: a clear bottom black plate and a plate reader capable of exciting at 335 nm and 536 nm and detecting emission at 512 nm and 617 nm
3. Flow cytometry: a cytometer equipped with a violet or ultraviolet laser and a blue (488 nm) laser

INTRODUCTION

Background

Autophagy is a critical cellular process that involves the degradation and digestion of intracellular components by the lysosome. This process not only enables cells to efficiently mobilize and recycle cellular constituents, but also prevents the accumulation of damaged organelles, misfolded proteins, and invading microorganisms.[1] Autophagy is a multi-step process that begins with the sequestration of cytoplasmic organelles and proteins. These cellular components are sequestered by a double membrane, forming an autophagosome. The autophagosome then fuses with a lysosome to form an autolysosome, where the cellular material is then degraded.[2,3] Normal autophagy is essential for survival, differentiation, development, and homeostasis. Dysregulation of autophagy has been implicated in cancer, infection, aging, and degenerative diseases.[2]

While autophagy most often acts to promote cell survival in response to stress, it can also promote cell death. The relationship between autophagy and apoptosis is complex. The two pathways share common stimuli and components, and can regulate the activity of each other.[4] However, the specific factors and mechanisms that dictate the choice between autophagy and apoptosis remain unclear.

About This Assay

Cayman's Autophagy/Cytotoxicity Dual Staining Kit provides a convenient tool for studying the regulation of autophagy and cytotoxicity at the cellular level. The kit employs monodansylcadaverine (MDC), a fluorescent compound that is incorporated into multilamellar bodies by both an ion trapping mechanism and the interaction with membrane lipids,[5] as a probe for detection of autophagic vacuoles in cultured cells. Propidium iodide is used as a marker of cell death. Tamoxifen, a known inducer of autophagy, is included as a positive control. This kit provides sufficient reagent to effectively treat/stain 960 individual wells of cells when utilized in a 96-well plate format.

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Cell-Based Assay Buffer Preparation**

   Dissolve each Cell-Based Assay Buffer Tablet (Item No. 10009322) in 100 ml of distilled water. This buffer should be stable for approximately one year at room temperature.

2. **Staining Solution Preparation**

   Bring the Cell-Based Monodansylcadaverine (MDC, Item No. 600141) to room temperature and vortex thoroughly before use. Prepare a staining solution by diluting both the MDC and the Cell-based Propidium Iodide Solution (PI, Item No. 10011234) 1:1,000 in the Assay Buffer prepared above. Mix well. Use immediately and protect from light.

General Precautions

MDC is extremely light sensitive and is photobleached quickly. All staining procedures must be performed without direct exposure to intense light. Therefore, incubations need to be done in the dark.

ASSAY PROTOCOL

Flow Cytometry

The following protocol is optimized for suspension cells treated and stained in 96-well plates. Adjust volumes accordingly for other sizes of plates. Optimal conditions will be dependent on the cell type.

1. Seed a 96-well plate with 0.5-2.5 x 10^5 cells/well.
2. Treat the cells with experimental compounds or vehicle control for the period of time used in your typical experimental protocol. To use the included Tamoxifen (Item No. 10011018) as a positive control, dilute 1:5,000-1:100,000 into your culture medium.
3. After the treatment period, transfer cells to a 96-well v-bottom staining plate, and centrifuge for five minutes at 400 x g at room temperature.
4. Remove the supernatant.
5. Add 100 µl of the Staining Solution, prepared as described on page 6, to each well. Incubate the cells for ten minutes at 37°C.
6. Centrifuge the plate for five minutes at 400 x g at room temperature and remove the supernatant.
7. Add 200 µl of Cell-Based Assay Buffer to each well.
8. Centrifuge the plate for five minutes at 400 x g at room temperature and remove the supernatant.
9. Add 100 µl of Cell-Based Assay Buffer to each well. The cells are now ready for analysis by flow cytometry and should be analyzed immediately. MDC is detectable using a violet (405 nm) or UV (350 nm) laser and filter at 525 nm. PI is excited by a 488 nm laser and detected by filters in the range of 650-700 nm.

Plate Reader or Fluorescence Microscopy Detection

The following protocol is designed for a 96-well plate and optimized for adherent cells. Adjust volumes accordingly for other sizes of plates.

1. Seed a 96-well black culture plate with 5 x 10^4 cells/well. Be sure to include extra wells for unstained controls. Grow cells overnight.
2. The next day, treat the cells with experimental compounds or vehicle control for 24-72 hours, or for the period of time used in your typical experimental protocol. To use the included Tamoxifen (Item No. 10011018) as a positive control, dilute 1:5,000-1:100,000 into your culture medium.

   NOTE: Differences in cell density can significantly affect results. Ensure that experimental compounds used do not significantly inhibit cell proliferation.
3. After the treatment period, centrifuge the plate for five minutes at 400 x g at room temperature.
4. Aspirate the supernatant.
5. Add 100 µl of the Staining Solution, prepared as described on page 6, to each well except the unstained control wells. Be careful to not disturb the cell layer. Incubate the cells for ten minutes at 37°C.
6. Centrifuge the plate for five minutes at 400 x g at room temperature.
7. Aspirate the supernatant.
8. Add 100 µl of Cell-Based Assay Buffer to each well. Be careful to not disturb the cell layer.
9. Centrifuge the plate for five minutes at 400 x g at room temperature.
10. Aspirate the supernatant.
11. Add 100 µl of Cell-Based Assay Buffer to each well. The cells are now ready for analysis and must be analyzed immediately. Autophagic vacuole staining intensity can be detected using an excitation wavelength of 335 nm and an emission wavelength of 512 nm or filter sets typically used to detect DAPI. The degree of cell death can be assessed by measuring PI staining intensity at excitation and emission wavelengths of 536 nm and 617 nm, respectively, or using filter sets typical to rhodamine or Texas Red detection.

PERFORMANCE CHARACTERISTICS

Cell Staining

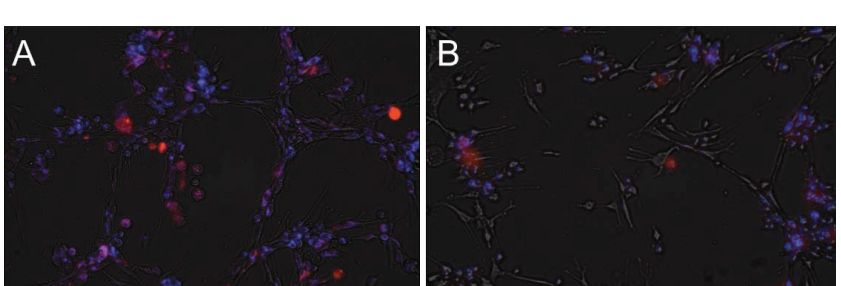


Figure 1. Tamoxifen increases autophagy in H9C2 cells as shown by fluorescence microscopy. H9C2 cells were seeded at 1 x 10^4 cells per well and incubated for 16 hours at 37°C/5% CO_2. Following incubation, cells were treated with vehicle, or 10 µM tamoxifen for 24 hours. After 24 hours cells were stained with MDC and propidium iodide using the staining solution described on page 6, and incubation times described on page 8. *Panel A* shows cells treated with 10 µM Tamoxifen. *Panel B* shows cell treated with vehicle. Images have been overlaid with to show red fluorescence (PI permeability), blue fluorescence (autophagic vacuoles), and bright field (cellular location). Cell images were obtained using the Cytation™ 5 Cell Imaging Multi-Mode Reader (BioTek Instruments, Inc.).

Flow Cytometry

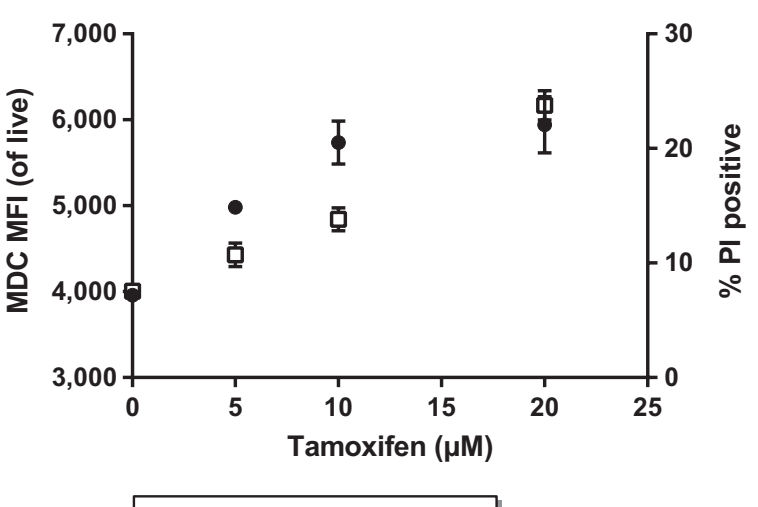


Figure 2. Tamoxifen increases autophagy and cell death in Jurkat cells. Jurkat cells were treated overnight with the indicated concentrations of tamoxifen, and stained with MDC and PI as described in the protocol on page 7. Data were collected on a MACSQuant cytometer (Miltenyi) and analyzed using FlowJo software (TreeStar). Shown are the geometric mean fluorescence intensity (MFI) of MDC staining in the live (PI negative) population, as well as the percentage of dead cells (% PI positive).

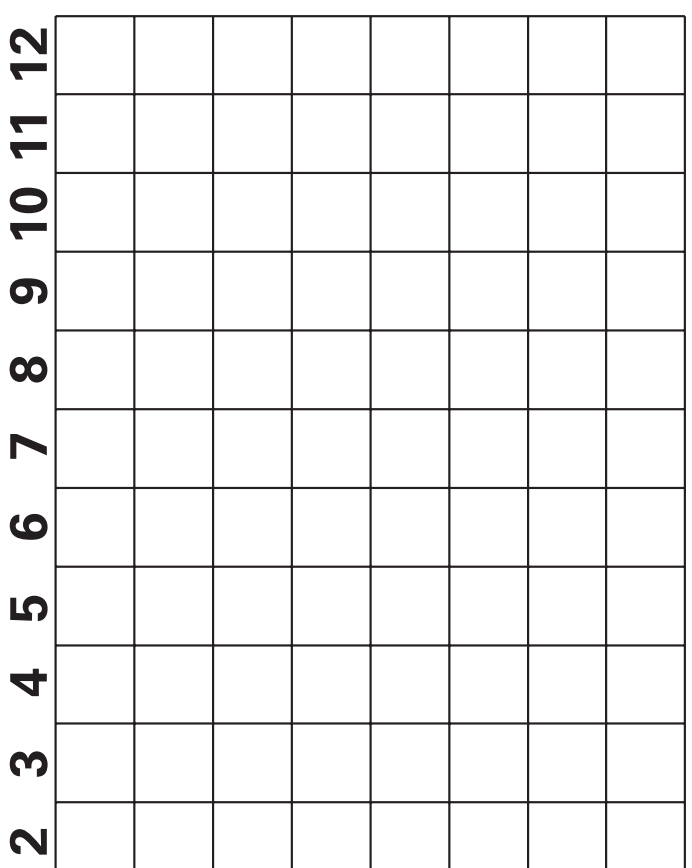
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Low MDC staining in all treatments, including positive control	Cells are not healthy	Use only healthy cells
High level of propidium iodide staining, including in control cells	Cells are not healthy or are dead	Use only healthy living cells
Low fluorescence intensity in both MDC and propidium iodide staining	A. Cell density too low B. Cells are lost during processing	A. Increase cell density B. Gently aspirate supernatant to ensure most of the cells stay on the plate

References

1. Jin, S. and White, E. Tumor suppression by autophagy through the management of metabolic stress. *Autophagy* **4(5)**, 563-566 (2008).
2. Levine, B. and Kroemer, G. Autophagy in the pathogenesis of disease. *Cell* **132**, 27-42 (2008).
3. Munafó, D.B. and Colombo, M.I. A novel assay to study autophagy: Regulation of autophagosome vacuole size by amino acid deprivation. *J. Cell Sci.* **114**, 3619-3629 (2001).
4. Mizushima, N., Levine, B., Cuervo, A.M., *et al.* Autophagy fights disease through cellular self-digestion. *Nature* **451**, 1069-1075 (2008).
5. Niemann, A., Takatsuki, A., and Elsässer, H.-P. The lysosomotropic agent monodansylcadaverine also acts as a solvent polarity probe. *J. Histochem. Cytochem.* **48(2)**, 251-258 (2000).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.