abcam

Product datasheet

Autophagy/Cytotoxicity Dual Staining Kit ab133075

2 References 2 Images

Overview

Product name	Autophagy/Cytotoxicity Dual Staining Kit
Sample type	Adherent cells
Assay type	Quantitative
Species reactivity	**Reacts with:** Mammals, Other species
Product overview	Autophagy/Cytotoxicity Dual Staining Kit (ab133075) 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, as a probe for detection of autophagic vacuoles in cultured cells. Propidium iodide (PI) 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. Lower density plates will still require approximately the same amount of reagent on a per plate basis. Therefore, up to 10 plates worth of cells can be examined irrespective of the number of wells/plate (this is not the case for protocols that use non-adherent cells).
Notes	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. 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. Normal autophagy is essential for survival, differentiation, development, and homeostasis. Dysregulation of autophagy has been implicated in cancer, infection, aging, and degenerative diseases. 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. However, the specific factors and mechanisms that dictate the choice between autophagy and apoptosis remain unclear.
Platform	Microplate reader, Fluorescence microscope

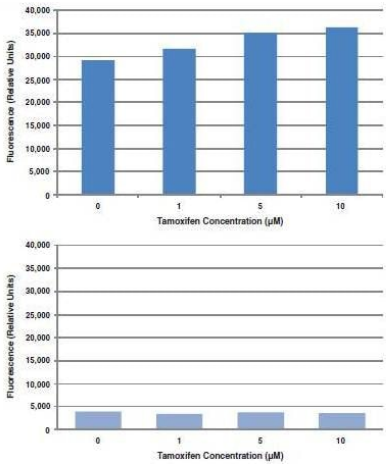
Properties

Properties

Storage instructions Please refer to protocols.

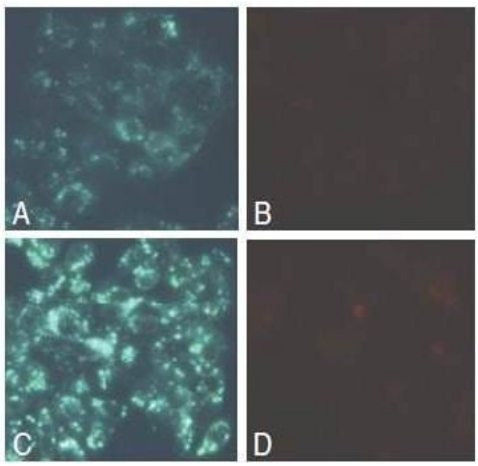
Components	1 kit	1 kit
Cell-Based Assay Buffer Tablet	5 tablets	5 tablets
Cell-Based Monodansylcadaverine	1 x 100µl	1 x 100µl
Cell-Based Propidium Iodide Solution	1 x 250µl	1 x 250µl
Cell-Based Tamoxifen (100 mM)	1 x 50µl	1 x 50µl

Images



Tamoxifen increases autophagy but not cell death in HepG2 cells.

HepG2 cells were seeded in a 96-well plate at a density of 5 x 10^4 cells/well in EMEM culture medium and incubated O/N at 37°C. The next day, cells were treated with increasing concentrations of tamoxifen and incubated O/N. On the third day, cells were stained with PI and MDC (as described in the assay protocol) and fluorescence was quantified using a plate reader.
Top panel: Tamoxifen treatment increases MDC fluorescence intensity, indicating that Tamoxifen treatment leads to an increase in autophagy in HepG2 cells.
Bottom panel: Tamoxifen treatment does not cause an increase in PI staining, indicating that at the concentrations used in this experiment, tamoxifen does cause cytotoxicity in HepG2 cells.



Tamoxifen increases autophagy but not cell death in HepG2 cells (fluorescence microscopy)

HepG2 cells were seeded at a density of 5 x 10^4 cells/well and incubated O/N at 37°C. The next day, cells were treated with either vehicle (panel A & B) or 10 µM of tamoxifen for 24 hours. On the third day, cells were stained with PI and MDC as described in the assay protocol.
Panel A: MDC staining of HepG2 cells treated with vehicle. There is a basal level of autophagy, indicated by faint silver dot staining of autophagic vacuoles. Panel B: PI staining of HepG2 cells treated with vehicle. There are few dead cells with only background staining of propidium iodide.
Panel C: MDC staining of HepG2 cells treated with 10 µM Tamoxifen. There is a clear increase in fluorescence intensity and number of autophagic vacuoles compared to the control cells treated with vehicle. Panel D: PI staining of HepG2 cells treated with 10 µM Tamoxifen, shows similar staining pattern to that of cells

treated with vehicle.

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