

Cell-ID™ Autophagy Detection Kit

for flow cytometry and fluorescence microscopy

Cell-ID™ Autophagy detection kit

ENZ-51031-K200

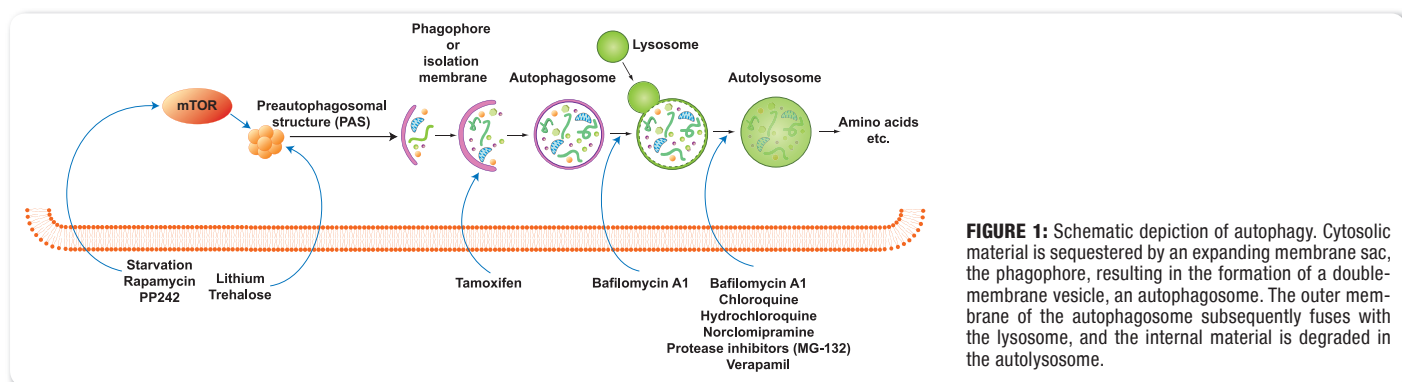
200 Assays



- Rapid, specific and quantitative approach for monitoring autophagy in live cells
- 488 nm laser excitable dye is compatible with a wide range of instruments, including conventional flow cytometers
- Validated under a wide range of conditions and with small molecule modulators known to influence autophagy pathways
- Optimized protocols for both fluorescence microscopy and flow cytometry applications
- Stringently manufactured, to control and eliminate non-specific assay artifacts

When subjected to certain hostile conditions that threaten survival, such as when extracellular nutrients are limiting, eukaryotic cells employ a lysosome-mediated intracellular bulk degradation pathway for digesting their own cellular contents by a process referred to as autophagy. Various cytoplasmic constituents, including organelles and long-lived proteins, are sequestered into double-membraned autophagosomes, which subsequently fuse with lysosomes where their contents are degraded. Autophagic activity is typically low under basal conditions, but can be markedly upregulated, both in cultured cells and intact organisms, by a variety of physiological stimuli such as nutrient starvation, hypoxia, energy depletion, endoplasmic reticulum stress, elevated temperature, high-density growth conditions, hormonal stimulation, pharmacological agent treatment, innate immune signaling, and in diseases such as viral, bacterial or parasitic infections as well as various protein aggregopathies, heart disease and acute pancreatitis.

Enzo Life Sciences' Cell-ID™ Autophagy detection kit provides a rapid, specific and quantitative approach for monitoring autophagy in live cells by fluorescence microscopy and flow cytometry. The 488 nm excitable green fluorescent detection reagent supplied in the Cell-ID™ Autophagy detection kit becomes brightly fluorescent in vesicles produced during autophagy and has been validated under a wide range of conditions known to modulate autophagy pathways. Tamoxifen (ALX-550-095), a known inducer of autophagy, is included as a positive control. A nuclear counterstain is provided in the kit to highlight this organelle as well. This live cell analysis kit provides a convenient approach for the analysis of the regulation of autophagy at the cellular level.



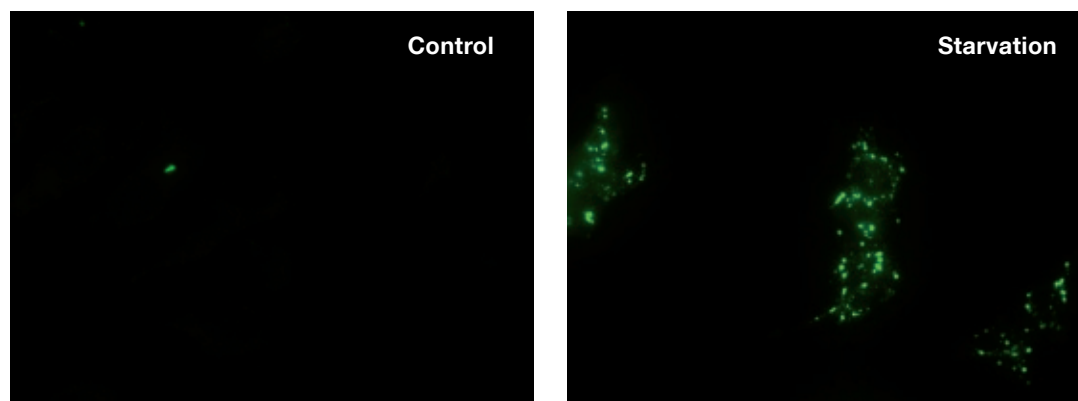


FIGURE 2: Cell-ID™ Green autophagy dye-labeled vesicles are induced by starvation: HepG2 cells were incubated in EMEM medium with 10% fetal bovine serum or in Earl's buffer (starvation) at 37°C for 1 h. Following this incubation period, both starved and control cells were incubated with Cell-ID™ Green Detection Reagent for 30 mins at 37°C and then washed with assay buffer. Cells were immediately imaged with a fluorescence microscope (Carl Zeiss, Inc) using an FITC filter set. Starvation induces an increase in fluorescence intensity in punctate cytoplasmic structures.

Treatment	Target	Effect
Starvation	Inhibits mammalian target of rapamycin (mTOR)	Activates autophagy
Rapamycin (BML-A275)	Inhibits mammalian target of rapamycin (mTOR)	Activates autophagy
PP242	ATP-competitive inhibitor of mTOR	Activates autophagy
Lithium	Inhibits IMPase and reduce inositol and IP ₃ levels; mTOR-independent	Activates autophagy
Trehalose	Unknown, mTOR-independent	Activates autophagy
Bafilomycin A1 (BML-CM110)	Inhibits vacuolar-ATPase	Inhibits autophagy
Chloroquine	Alkalinizes lysosomal pH	Inhibits autophagy
Tamoxifen (ALX-550-095)	Increases the intracellular level of ceramide and abolishes the inhibitory effect of PI3K	Activates autophagy
Verapamil (ALX-550-306)	Ca ²⁺ channel blocker; reduces intracytosolic Ca ²⁺ levels; mTOR-independent	Activates autophagy
Hydroxychloroquine	Alkalinizes lysosomal pH	Inhibits autophagy
Loperamide (ALX-550-253)	Ca ²⁺ channel blocker; reduces intra-cytosolic Ca ²⁺ levels; mTOR-independent	Activates autophagy
Clonidine (ALX-550-087)	Imidazoline-1 receptor agonist; reduces cAMP levels; mTOR-independent	Activates autophagy
MG-132 (BML-PI102)	Selective proteasome inhibitor	Activates autophagy
Norclomipramine	Alkalinizes lysosomal pH	Inhibits autophagy

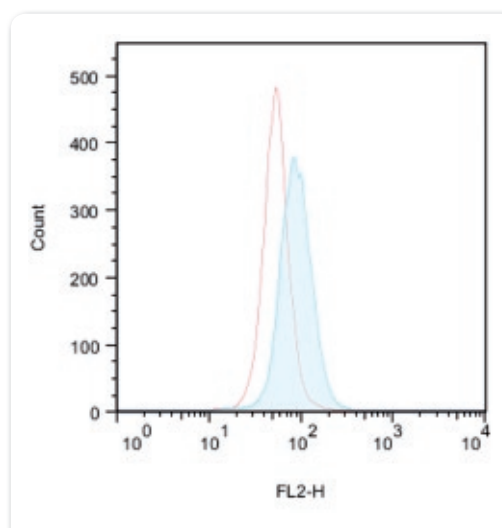


FIGURE 3: Flow cytometry-based profiling of autophagy with Cell-ID™ Autophagy Detection Kit: Control (red-lined peak) uninduced and 10 μM Tamoxifen (ALX-550-095) treated (blue-filled peak) Jurkat cells (T-Cell leukemia) were used. After 18 hours treatment, cells were loaded with Cell-ID™ Green Detection Reagent, then analyzed without washing by flow cytometry. Results are presented by histogram overlays. Control cells were stained as well but mostly display low fluorescence. In the samples treated with 10 μM Tamoxifen for 18 hours, Cell-ID™ Green dye signal increases about 2-fold, indicating that Tamoxifen causes an increase in autophagy in Jurkat cells.

Related Products

Product	Prod. No.	Size
Lyso-ID® Red cytotoxicity kit (GFP-Certified™)	ENZ-51015-KP002	2 x 96 wells

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