CYTO-ID® Autophagy detection kit

A no-transfection, quantitative assay for monitoring autophagy in live cells

Enzo Life Sciences CYTO-ID® Autophagy Detection Kit measures autophagic vacuoles and monitors autophagic flux in lysosomally inhibited live cells using a novel dye that selectively labels accumulated autophagic vacuoles. The 488nm-excitable green dye has been optimized through the identification of titratable functional moieties that allow for minimal staining of lysosomes while exhibiting bright fluorescence upon incorporation into pre-autophagosomes, autophagosomes, and autolysosomes (autophagolysosomes). The kit also includes the Hoechst 33342 dye for the nuclear staining, an Autophagy Inducer (Rapamycin) and a Lysosomal Inhibitor (Chloroquine).

Mechanism of Action
The probe is a cationic amphiphilic tracer (CAT) dye that rapidly partitions into cells in a similar manner as drugs that induce phospholipidosis. Careful selection of titratable functional moieties on the dye prevents its accumulation within lysosomes, but enables labeling of vacuoles associated with the autophagy pathway.

Autophagy is a stress-induced protective mechanism. Less active under basal conditions, the mechanism is utilized by eukaryotic cells through lysosome-mediated bulk degradation of cellular contents when subjected to certain hostile conditions such as nutrient depletion and chemical or environmental stress. The role of increased autophagic activity in the pathology of cancer, neurodegeneration, cardiovascular disease and diabetes has become widely recognized and commonly studied. Induction of autophagic flux can be visualized by enhanced accumulation of autophagic vesicles if lysosomal function is inhibited, preventing removal of these vesicles.

Citations: 449 View Online »

- Rapid, no transfection required
- Protocol validated with known inhibitors and activators of autophagic activity
- Selective and comprehensive staining allows differentiation between autophagic flux and autophagolysosome accumulation
- Negligible staining of lysosomes reduces background seen with other dyes
- Facilitates high-throughput screening of activators and inhibitors of autophagy

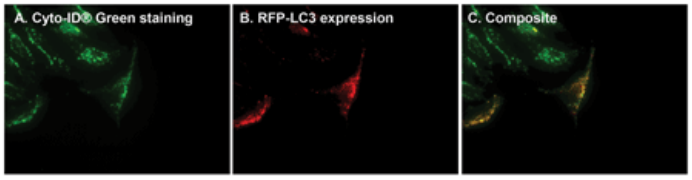
Ordering Information

Order Online »

ENZ-51031-0050	50 tests
ENZ-51031-K200	200 tests

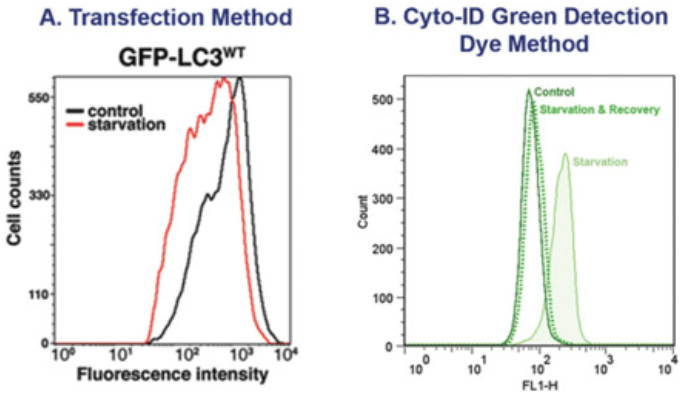
Manuals, SDS & CofA

View Online »

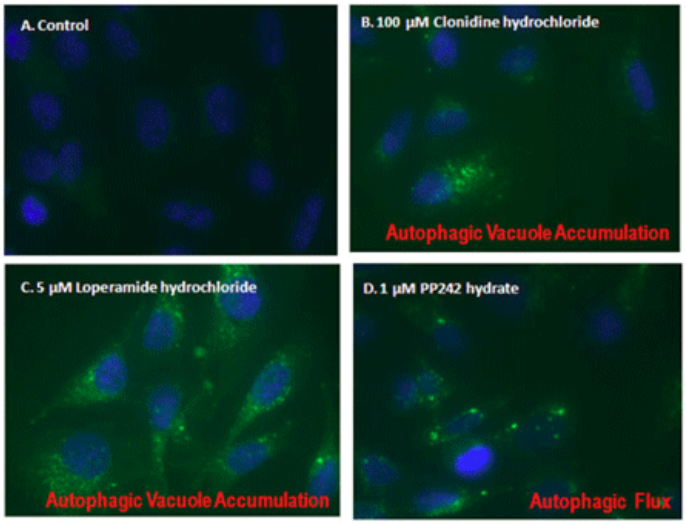


Time-saving, rapid and comprehensive labeling of autophagic vacuoles without transfection. For the purpose of demonstrating advantages of CYTO-ID® Green detection reagent, HeLa cells were first transfected with RFP-LC3 expression vector, treated with 10 µM Tamoxifen overnight, then stained with CYTO-ID® Green detection reagent. Unlike overnight transfection-based assays, the CYTO-ID® Green detection reagent approach labels 100% of cells in 15-30 minutes. Panel A: Green signal indicating CYTO-ID® Green staining of autophagic vesicles; Panel B: RFP-LC3 expression (red) in a subset of successfully transfected cells; Panel C: Composite image, showing CYTO-ID® Green dye-labeled vesicles co-localize with LC3, a specific marker of autophagosomes.

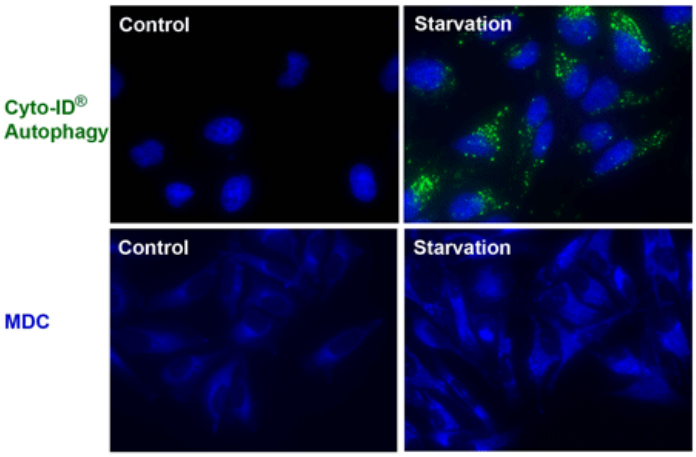
CYTO-ID® Autophagy Detection Kit



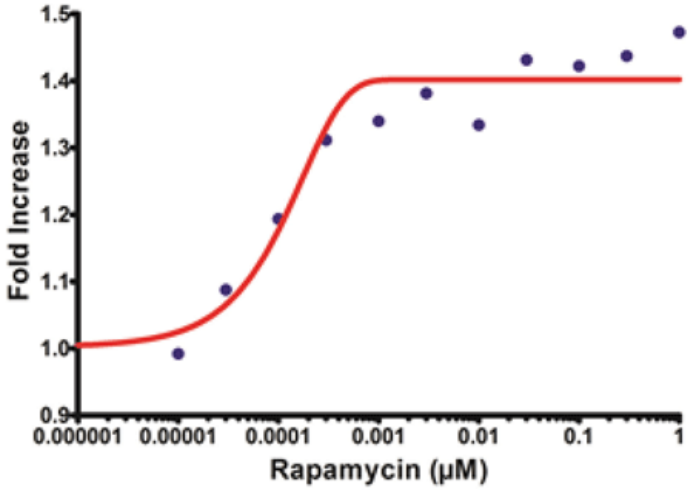
Profile autophagy without transfection. Figure 1A: CHO cells stably expressing GFP-LC3 transfected cell lines results in relatively poor baseline separation of control-vs-starved cell populations, making quantification of autophagy difficult. Figure adapted from Shvets E, Fass E, Elazar Z. Figure 1B: The CYTO-ID® Autophagy Detection Kit specifically labels autophagic vacuoles independent of LC3 protein and eliminates the need for transfection. HeLa cells were subjected to starvation and recovery and then labeled with CYTO-ID® Green detection reagent. The dye enables clear detection and quantification of autophagic and pre-autophagic vacuoles that directly correlates to induction of autophagy.



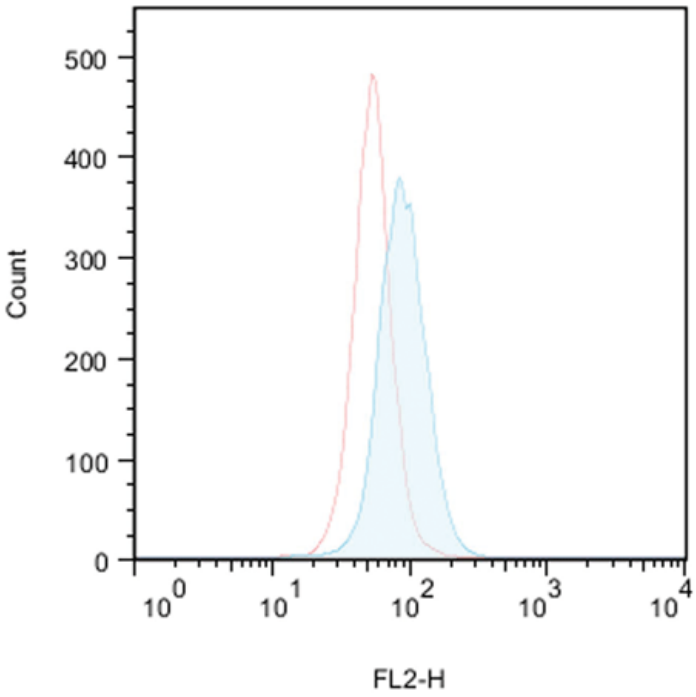
Visualization of autophagic accumulation and autophagic flux. Autophagic vacuole accumulation and flux are both detected by CYTO-ID® Autophagy Green dye as observed by fluorescence microscopy. HeLa cells were mock-induced with 0.2% DMSO (A) or induced with 100 uM Clonidine hydrochloride (B), 5 uM Loperamide hydrochloride (C) or 1 uM PP242 hydrate (D) for 12 hours at 37°C. After treatment, cells were incubated with CYTO-ID® Green Detection reagent for 10 min at 37°C and then washed with assay buffer. Nuclei were counter-stained in blue with Hoechst 33342 dye.



Eliminate background resulting from non-specific lysosomal staining. CYTO-ID® Green dye eliminates background staining of lysosomes seen with other lysosomotrophic dye-based assays that utilize monodansylcadaverine (MDC) (bottom panel). The CYTO-ID® Autophagy kit eliminates the need for a 350 nm UV laser for live cell analysis, and is compatible for use with Hoechst dyes for co-labeling in microscopy applications.



Overnight incubation of HepG2 cells with Rapamycin, an inhibitor of mTOR kinase, results in an increase in CYTO-ID® dye signal.



Flow cytometry-based profiling of autophagy with CYTO-ID® Autophagy Detection Kit: Control (red-lined peak) uninduced and 10uM Tamoxifen (ALX-550-095) treated (blue-filled peak) Jurkat cells (T-cell leukemia) were used. After 18 hours treatment, cells were loaded with CYTO-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 10uM Tamoxifen for 18 hours, CYTO-ID® Green dye signal increases about 2-fold, indicating that Tamoxifen causes an increase in autophagy in Jurkat cells.

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

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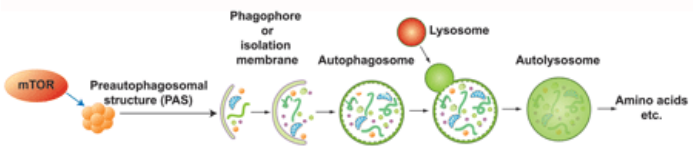

Schematic depiction of autophagy. Cytosolic material is sequestered by an expanding membrane sac, the phagophore, resulting in the formation of a double-membrane vesicle, an autophagosome. The outer membrane of the autophagosome subsequently fuses with the lysosome, and the internal material is degraded in the autolysosome. Various regulators of autophagy are also depicted in the diagram.

Handling & Storage

Use/Stability	With proper storage, the kit components are stable for one year from date of receipt.
Handling	Protect from light. Avoid freeze/thaw cycles.
Short Term Storage	-20°C
Long Term Storage	-80°C
Shipping	Blue Ice

Regulatory Status

RUO - Research Use Only

Product Details

Application	Flow Cytometry, Fluorescence microscopy, Fluorescent detection, HTS
Application Notes	The CYTO-ID® Autophagy detection kit provides a rapid, specific and quantitative approach for monitoring autophagy in live cells by fluorescence microscopy, flow cytometry, and microplate reader.
Contents	CYTO-ID® Green Detection Reagent Hoechst 33342 Nuclear Stain Autophagy Inducer (Rapamycin) Chloroquine Control 10X Assay Buffer
Quality Control	A sample from each lot of CYTO-ID® Autophagy detection kit is used to stain HeLa Cells as described in user manual. CYTO-ID® autophagy detection reagent is incorporated into induced cells, observed as accumulative typical spherical vacuoles in foci or throughout cytoplasm. Comparing to untreated HeLa cells, treated sample demonstrate significant increase in fluorescence under microscope.
Quantity	For -K200 size: 200 flow cytometry assays, 250 microscopy assays or 3 x 96-well microplate assays. For -0050 size: 50 flow cytometry assays, 60 microscopy assays or 1 x 96-well microplate assays.

Technical Info / Product Notes

The CYTO-ID® Autophagy Detection kit is a member of the CELLESTIAL® product line, reagents and assay kits comprising fluorescent molecular probes that have been extensively benchmarked for live cell analysis applications.

Featured in:

Genetic Engineering & Biotechnology News – *HTS Profiling Method for Autophagy-Modulators*

Application Notes:

Autophagy Analysis Using Object Spot Counting Using Gen5 to Analyze the Size and Number of Autophagosomes Per Nuclei

Towards Understanding the Molecular Basis of Parkinson's Disease: Cell-based Model of Mitophagy and Aggresome Accumulation

Response Profiles of Known Autophagy-Modulators Across Multiple Cell Lines: Using CYTO-ID® Autophagy Dye to assess Compound Activity and Toxicity

Cell-Based Screening of Focused Bioactive Compound Libraries: Assessing Small Molecule Modulators of the Canonical Wnt Signaling and Autophagy-Lysosome Pathways

A Novel Image-Based Cytometry Method for Autophagy Detection in Living Cells

Predictive High-Content/High-Throughput Assays for Hepatotoxicity Using Induced Pluripotent Stem Cell (iPSC)-Derived Hepatocytes

Visualizing subcellular vesicles to quantitate autophagy in neuronal cells

Cited samples:

For an overview on cited samples please click here.

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