CYTO-ID® Autophagy detection kit 2.0

A brighter and more photostable, no-transfection, quantitative assay for monitoring autophagy in live cells

Enzo Life Sciences CYTO-ID® Autophagy Detection Kit 2.0 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.

- Brighter, more photostable dye specifically stains autophagic vesicles
- Rapid, no transfection required
- Negligible staining of lysosomes reduces background seen with other dyes
- Facilitates high-throughput screening of activators and inhibitors of autophagy
- Widely cited

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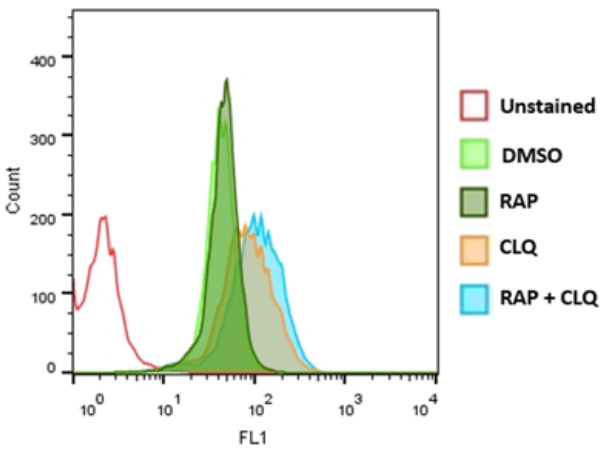
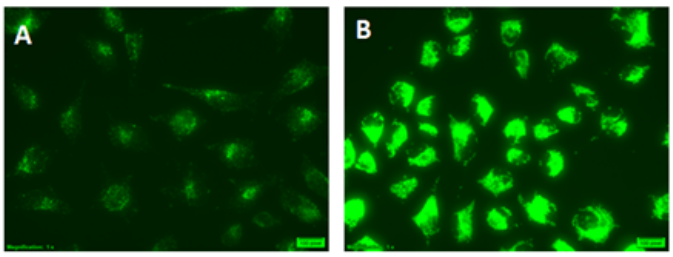
ENZ-KIT175-0050	50 tests
ENZ-KIT175-0200	200 tests

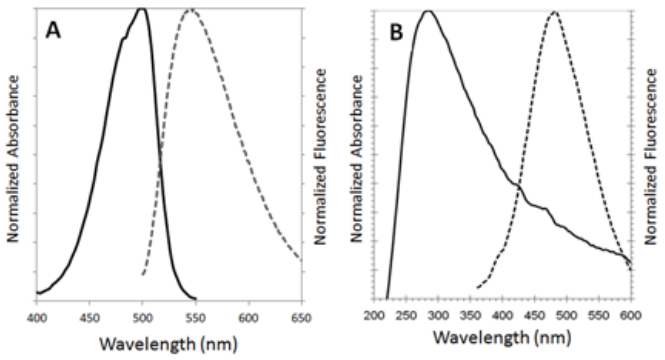
Manuals, SDS & CofA View Online »



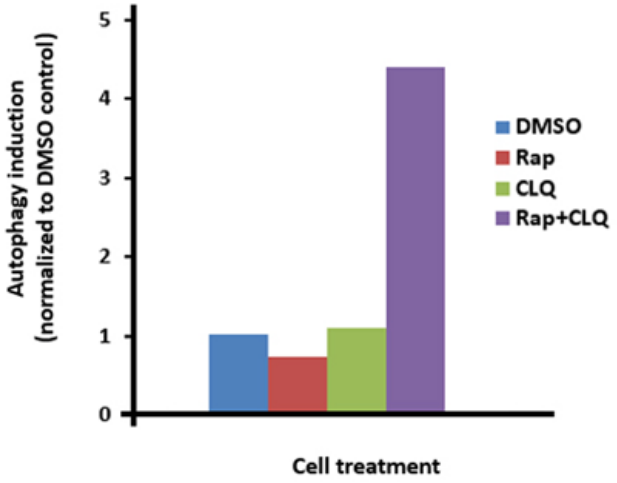
Flow cytometry-based profiling of autophagy in Jurkat cells. Jurkat cells were untreated or treated with 0.5μM Rapamycin (RAP), 10μM Chloroquine (CLQ) or both for 20h. After staining with CYTO-ID® Green Detection Reagent 2 for 30min, cells were washed and analyzed by flow cytometry. Results are presented as histogram overlay. Cells treated with RAP + CLQ show an increase in fluorescence.



HeLa cells were stained with CYTO-ID® Green Detection Reagent 2 after being cultured in (A) full media or (B) starvation media (EBSS) with 40μM Chloroquine for 4h. Cells starved in EBSS in the presence of Chloroquine showed very bright green fluorescent signals and punctate structures.



Absorbance and fluorescence emission spectra (499/548nm) for CYTO-ID® Green Detection Reagent 2 (panel A) were determined in PBS. Absorbance and fluorescence emission spectra (350/461nm) for Hoechst 33342 (panel B) were determined in 1X Assay Buffer.



Microplate-based profiling of autophagy in HepG2 cells. HepG2 cells were stained CYTO-ID® Green Detection Reagent 2 after being cultured for 20h in DMSO (control), 0.5 µM Rapamycin (Rap), 10µM Chloroquine (CLQ), or both 0.5µM Rap and 10µM CLQ. Cells were also stained with Hoechst 33342 for cell number normalization. Cells treated with both Rapamycin and Chloroquine had an increase in autophagy, as measured by the CYTO-ID® Green Detection Reagent 2.

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 2.0 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 2 Hoechst 33342 Nuclear Stain Autophagy Inducer (Rapamycin) Chloroquine Control 10X Assay Buffer
Quality Control	Microscopy: When CYTO-ID® Green autophagy detection reagent 2 is incorporated into cells, the accumulation of this fluorescent probe is typically observed in spherical vacuoles in the perinuclear region of the cell, in foci distributed throughout the cytoplasm, or in both locations, depending upon the cell type under investigation. The un-induced cells show very low background staining comparing to autophagic samples prepared as described in the user manual.

Quantity

For -0200 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 2.0 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.

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