

# Lyso-ID<sup>®</sup> Red Cytotoxicity Kit

## for microplates (GFP-Certified<sup>™</sup>)

A variety of cell types are known to respond to cationic amphiphilic drugs (CADs) and other basic compounds by the formation of multiple large vacuoles, which have been variously referred to as lamellar bodies or lysosomal inclusion bodies.

The persistence of giant autophagosome structures in cells is thought to be due to the retention of the cationic drugs by ion trapping, as well as inhibition of phospholipases.

Phospholipidosis is the intracellular accumulation of phospholipid-rich membranes in vacuoles, often triggered by such CADs as procainamide, verapamil and chloroquine, which cytopathologically sequester within the cells. Several mechanisms have been proposed to explain this accumulation, including drug-phospholipid complexes' resistance to degradation by phospholipases, direct inhibition of phospholipases and inhibition of intracellular pathways regulating phospholipid metabolism.

Unlike conventional lysosome stains, this kit is effective for detecting drug-induced phospholipidosis in live cells induced by CADs. In addition, agents that cause the accumulation of autophagosomes by blocking the downstream lysosomal pathway and/or intracellular trafficking of autophagosomes also lead to increases in the accumulation of intracellular Lyso-ID<sup>®</sup> Red dye signal in the described assay. This microplate assay offers several advantages over alternative methods based upon electron microscopy, fluorescence microscopy, flow cytometry or long term incubation with fluorescent phospholipid analogs.

Chief among these advantages are the ability to analyze drug response in cells without co-incubation with artificial phospholipid analogs and the ability to perform drug screening in a rapid and quantitative high-throughput manner using a conventional fluorescence microplate reader. A lysosome-perturbation agent, verapamil, is provided as a positive control and a blue nuclear counterstain is integrated into the detection reagent to identify cell death.

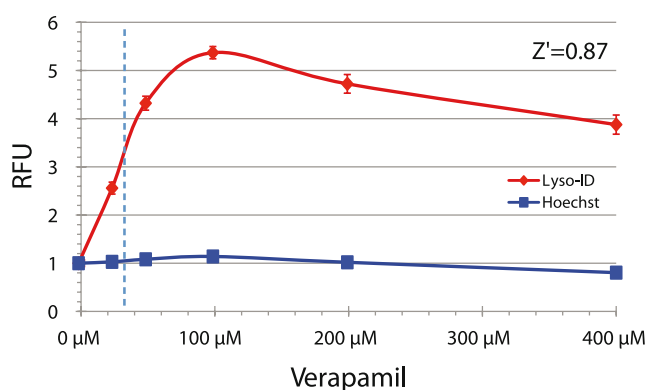
## Lyso-ID<sup>®</sup> Red Cytotoxicity Kit (GFP-Certified<sup>™</sup>)

ENZ-51015-KP002

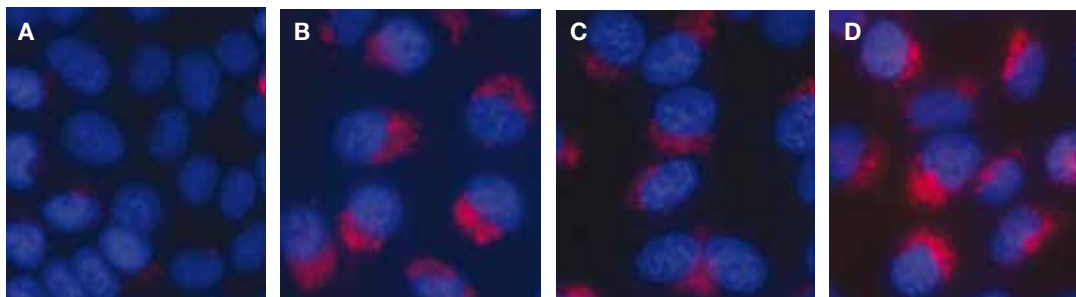
2 x 96 wells

Lyso-ID<sup>®</sup> Red cytotoxicity kit (GFP-Certified<sup>™</sup>) for microplates is a 96-well cell-based assay that provides a rapid and quantitative approach for determining drug- or toxic agent-induced lysosome and lysosome-like organelle perturbations in live cells.

- Monitors dysfunction of lysosomal degradation
- Analyze drug response in cells without co-incubation with artificial phospholipid analogs
- Applicable to *in vitro* toxicology and preclinical drug safety assessment
- Rapid (10-15 minute) dye incubation
- No fixation or permeabilization steps



**FIGURE: Screen compounds for lysosome-perturbing activity.** Using a conventional fluorescence microplate reader, the half maximal effective concentration ( $EC_{50}$ ) of verapamil in U2OS cells was estimated. The high Z-factor (0.87 for 100µM verapamil) obtained using the assay demonstrates excellent signal-to-noise and signal-to-background ratios. The error bars denote the standard deviation of at least six determinations.



**FIGURE: Changes in the lysosomal compartment arising from phospholipidosis.** Fluorescent microscopy images of untreated U2OS cells (A), Chloroquine-treated cells (B), Chlorpromazine-treated cells (C), and Verapamil-treated cells (D). These three compounds are cationic and amphiphilic, and known to induce abnormal accumulation of phospholipids within lysosomes, resulting in lamellar bodies. Nuclei are counter-stained with Hoechst 33342 dye.

## Ordering Information

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

## Related Products

Product	Prod. No.	Size
<b>Mito-ID® Membrane potential cytotoxicity kit</b>	ENZ-51019-KP002	2 x 96 wells
<b>Mito-ID® Membrane potential detection kit</b>	ENZ-51018-K100	100 Assays
<b>Nuclear-ID® Green chromatin condensation kit</b>	ENZ-51021-K200	200 Assays

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