

## Autophagy Screening on iQue<sup>®</sup>

### SUMMARY

The iQue Screener is an excellent platform for screening for perturbations to autophagy in a rapid no wash screen-ready assay.

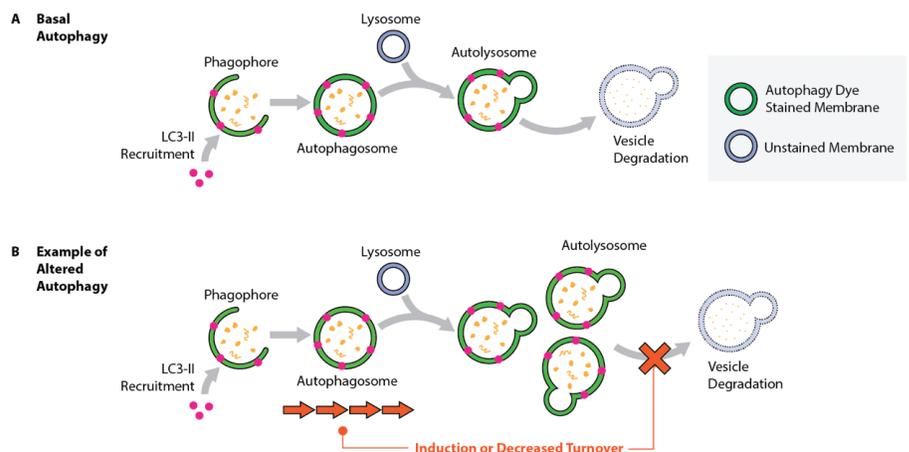
- Enzo's Cyto-ID<sup>®</sup> Autophagy detection kit on the iQue Screener makes a fast and easy screening assay for perturbations to the amount of autophagic vesicles
- See the effect of 14 known effectors of autophagy and viability on 6 difference cell lines below
- Like IntelliCyt's MultiCyt<sup>®</sup> reagents, this Enzo dye performs well on the iQue with a no wash protocol
- Go from sample preparation to data analysis in 1 hour for a 384-well plate

### PROBLEM

Autophagy is an important and complex biological process by which cells turn over proteins and organelles in a targeted manner. This process is routine in maintaining homeostasis in healthy cells, but is also implicated in disease states such as cancer, infection, and neurodegeneration. The process is understood to initiate with the formation of an autophagosome that surrounds components in the cytoplasm and then fuses with a lysosome to create an autolysosome. The contents of these vesicles are enzymatically degraded. Increases or decreases to this activity are problematic for the cells. Common assay for autophagy, such as imaging and Western blots, are slow and usually require genetic modification (GFP-LC3 fusions) to get a measurable endpoint.

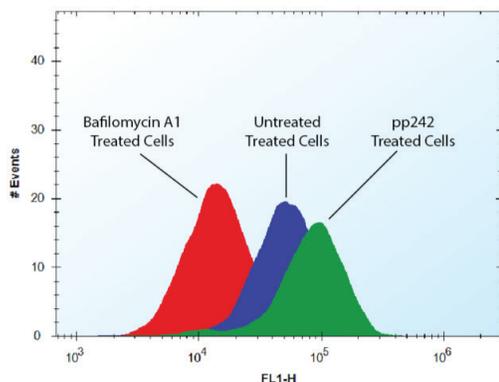
### RESULTS

Enzo's Cyto-ID Autophagy Detection Kit detects modulation of autophagy by selectively staining membranes of phagophores, autophagosomes, and autolysosomes, with minimal staining of lysosomes.

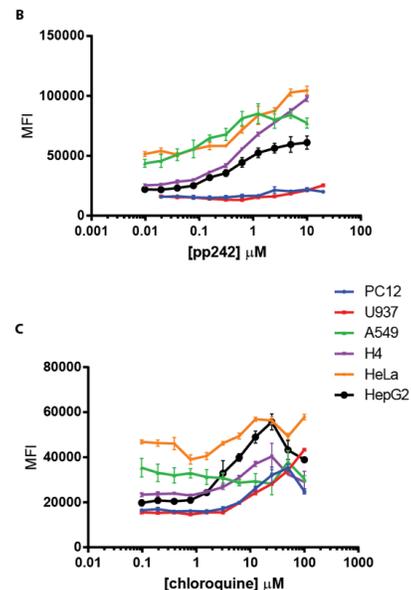
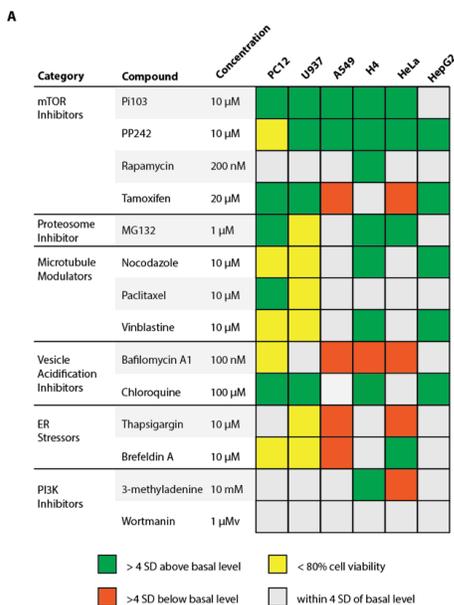


**Figure 1.** (A) Mammalian cells undergo a basal level of autophagy as a normal homeostatic process and will stain positively with Cyto-ID Autophagy Dye. (B) Any perturbation that causes a change to the total amount of autophagic compartments will cause cells to be stained differentially than untreated cells. The accumulation or depletion of autophagic compartments can be caused by either upregulation or inhibition of steps in the autophagy pathway. Because of this, the Cyto-ID Autophagy Dye is a gross measure of autophagy perturbation rather than a specific reporter of the magnitude of change to autophagic-flux.

Cyto-ID Autophagy Dye enables a simplified, no-wash workflow when used with the iQue Screener. After performing compound treatments, the Cyto-ID Autophagy Dye is added directly to the wells of your treatment plates. After a 30 min incubation at 37°C, plates are read directly on the iQue Screener in about 25 minutes for a 384-well plate.



**Figure 2.** Known modulators of autophagy cause changes in Cyto-ID Autophagy Dye staining of A549 cells. The frequency distribution of Autophagy Dye staining intensity is shown for A549 cells treated with Bafilomycin A1 (red), pp242 (green), and untreated cells (blue). Treatment with pp242 causes increased staining of cells relative to untreated cells. Treatment with Bafilomycin A1 causes decreased staining of cells relative to untreated cells.



**Figure 3.** Response profiles for 14 known autophagy-modulators. A) A response was considered significant if it caused Cyto-ID Autophagy Dye staining to change more than 4 standard deviations from untreated cells. Responses included both increased staining (green boxes) and decreased staining (red boxes). Cyto-ID Autophagy Dye responses were not scored when treatments that caused cell viability to drop below 80% (yellow boxes). Dose response treatment with pp242 (B) or chloroquine (C) in 6 different cell lines.

### THE INTELLICYT SOLUTION

- Using Enzo’s Cyto-ID Autophagy Detection kit on the iQue Screener is a fast and easy way to screen for modulators of autophagy in both adherent and suspension cells
- MultiCyt Membrane Integrity (and potentially Cell Proliferation and Cell Cycle) dye can be multiplexed with Cyto-ID Autophagy Detection dye for single well high content profiling
- The no wash assay plates are read in about 25 minutes for 384-well plate on the iQue.

Visit [www.intellicyt.com](http://www.intellicyt.com) to learn how iQue can improve your screens!



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