Cell Meter™ Autophagy Fluorescence Imaging Kit

Blue Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 23001 (200 assays)	Keep in freezer Avoid light	Fluorescence microscope, flow cytometer, and fluorescence microplate reader

Introduction

Autophagy is one of the major pathways for degradation of intracellular macromolecules in animal cells. The process of autophagy involves the sequestration of cytoplasmic materials and intracellular organelles in a membrane-bounded vacuole called autophagosome, the fusion of the autophagosome with lysosomes, and the subsequent degradation of sequestered materials. Cell Meter™ autophagy fluorescence imaging kit employs Autophagy Super Blue™ as a specific autophagosome marker to analyze the activity of autophagy. The assay is optimized for direct detection of autophagy in both detached and attached cells. The kit provides all the essential components for the assay protocol. Cell Meter™ autophagy fluorescence imaging kit is optimized for fluorescence microscope, it is also suitable for flow cytometer. Autophagy Super Blue™ has fluorescence excitation and emission at Ex/Em = 333/518 nm.

Kit Components

Components	**Amount**
Component A: 500X Autophagy Super Blue™	50 µL
Component B: Stain Buffer	25 mL
Component C: Wash Buffer	100 mL

Assay Protocol for One 96-Well Plate (adherent cells):

Brief Summary

Prepare cells with your test compounds at the density of 1-2 × 10^4 cells/well → Add Autophagy Super Blue™ working solution→ Incubate at 37ºC for 15 min-1 hour → Wash cells with Wash Buffer → Analyze the cells at Ex/Em = 335/520 nm

Note: Thaw all the components at room temperature before use.

1. Culture cells to a density optimal for autophagy induction according to your specific induction protocol (about 1-2 × 10^4 cells/ well/96-well plate). At the same time, culture a non-induced negative control cell population at the same density as the induced population for every labeling condition.

2. Prepare Autophagy Super Blue™ working solution by diluting 20 µL of Autophagy Super Blue™ (Component A) to 10 mL of Stain Buffer (Component B).
Note: 20 µL of 500 X Autophagy Super Blue™ (Component A) is enough for one 96-well plate. Aliquot and store unused 500 X Autophagy Super Blue™ at ≤ -20ºC. Protect from light and avoid repeated freeze-thaw cycles.

3. Remove medium, add 100 µL of Autophagy Super Blue™ working solution (from Step 2) into each well, and incubate the cells in a 37ºC, 5% CO_2 incubator for 15 min-1 hour.
   Note: The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.

4. Wash the cells with Wash Buffer (Component C) for 3-4 times, add 100 µL Wash Buffer (Component C) to each well.
   Note: It is recommended to increase either the labeling concentration or the incubation time to allow the dye to accumulate if the cells do not appear to be sufficiently stained.

5. Monitor the fluorescent intensity with a fluorescence microscope or flow cytometer at Ex/Em = 330/520 nm.

Data Analysis

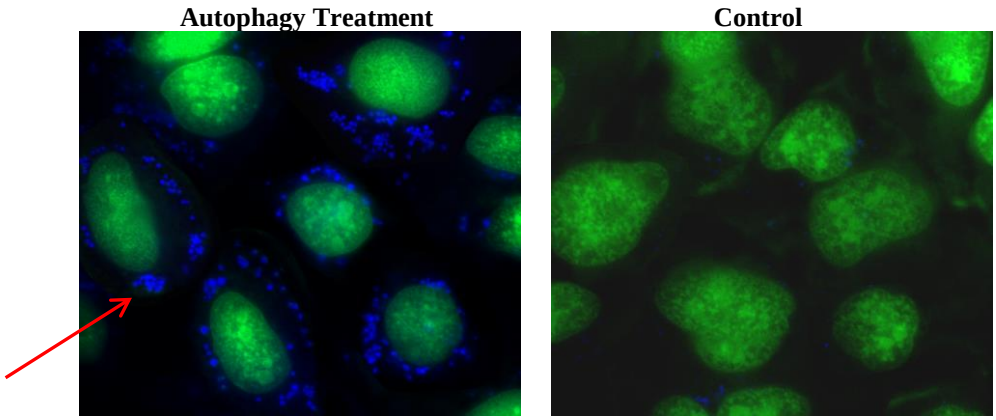

Figure 1. Autophagy Super Blue™ labeled vesicles were induced by starvation in HeLa cells. HeLa cells were incubated in a regular DMEM medium (left: Control) or in 1X HBSS buffer with 5% serum (Left: Autophagy Treatment) for 16 hours. Both control cells and treated cells were incubated with Autophagy Super Blue™ working solution for 20 minutes in a 37 °C, 5% CO_2 incubator, and washed 3 times with wash buffer. Cells were imaged immediately under a fluorescence microscope with a DAPI channel (blue). Cell nuclei were stained with Nuclear Green™ LCS1 (Cat#17540, green).

References

1. Munafó D.B. and Colombo M.I. (2001) A novel assay to study autophagy: regulation of autophagosome vacuole size by amino acid deprivation. J Cell Sci. 114:3619-3629.
2. Raben N., Shea L., Hill V., Plotz P. (2009) Monitoring autophagy in lysosomal storage disorders. Methods Enzymol.,453:417-49.
3. Niemann A. (2000) The lysosomotropic agent monodansylcadaverine also acts as a solvent polarity probe. J Histochem Cytochem 48:251.