Cell Meter™ Autophagy Fluorescence Imaging Kit

Catalog number: 23001
Unit size: 200 Tests

Component	Storage	Amount
Component A: 500X Autophagy Super Blue™	Freeze (<-15 °C), Minimize light exposure	1 vial (50 µL)
Component B: Stain Buffer	Freeze (<-15 °C), Minimize light exposure	1 bottle (25 mL)
Component C: Wash Buffer	Freeze (<-15 °C), Minimize light exposure	100 mL

OVERVIEW

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. The Cell Meter™ autophagy fluorescence imaging kit is optimized for fluorescence microscope. It gives much higher selectivity than other commercially available autophagy probes.

AT A GLANCE

Protocol summary

1. Prepare cells with your test compounds at the density of 1 - 2 × 10^4 cells/well
2. Add Autophagy Super Blue™ working solution
3. Incubate at 37°C for 15 - 60 minutes
4. Wash cells with Wash Buffer
5. Monitor the fluorescence increase at Ex/Em= 330/520 nm (Cutoff = 475 nm), fluorescence microscope with DAPI filter set

Important Thaw all the components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	DAPI filter
Emission:	DAPI filter
Recommended plate:	Black wall/clear bottom
Instrument:	Fluorescence microplate reader
Excitation:	330 nm
Emission:	520 nm
Cutoff:	475 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Bottom read mode

PREPARATION OF WORKING SOLUTION

Add 20 µL of 500X Autophagy Super Blue™ (Component A) to 10 mL of Stain Buffer (Component B) and mix well to make Autophagy Super Blue™ working solution. Protect from light.

Note 20 µL of 500X Autophagy Super Blue™ (Component A) is enough for one 96-well plate.

SAMPLE EXPERIMENTAL PROTOCOL

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. Remove medium.
3. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Autophagy Super Blue™ working solution into each well.
4. Incubate the cells in a 37°C, 5% CO_2 incubator for 15 to 60 minutes.

   Note The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.
5. Wash the cells with Wash Buffer (Component C) for 3 - 4 times, then 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.
6. Monitor the fluorescence intensity with a fluorescence microplate reader at Ex/Em = 330/520 nm (Cutoff = 475 nm), a fluorescence microscope with DAPI filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

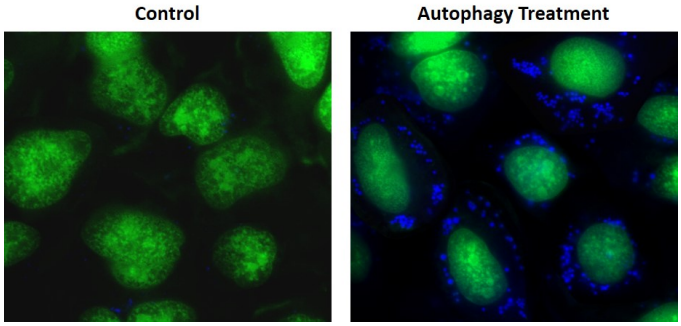

Figure 1. Autophagy Super Blue™ labeled vesicles were induced by starvation in HeLa cells. HeLa cells were incubated in a regular DMEM medium (Left: Control) or in 1X HBSS buffer with 5% serum (Right: Autophagy Treatment) for 16 hours. Both control and treated cells were incubated with Autophagy Super Blue™ working solution for 20 minutes in a 37 °C, 5% CO2 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 (green).

DISCLAIMER

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