Cell Meter™ Autophagy Assay Kit *Blue Fluorescence*

Catalog number: 23000
Unit size: 200 Tests

Component	Storage	Amount
Component A: 500X Autophagy 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 an evolutionarily conserved degradation process that targets long-lived proteins, organelles, and other cytoplasmic components for degradation via the lysosomal pathway. The autophagy pathway is complementary to the action of the ubiquitin-proteasome pathway which typically degrades short-lived proteins. Activation of the autophagy pathway is required for multiple cellular roles, including survival during starvation, the clearance of intracellular components, development, and immunity. In the absence of stress, autophagy serves a house-keeping function, removing damaged organelles and cellular components preventing cytotoxic effects. Decreases and defects in autophagy have been implicated in multiple diseases, for example Huntingtons, Alzheimers, and Parkinsons. In terms of cancer development, autophagy seems to play multiple roles. Decreased or absent expression of certain autophagy proteins, such as Beclin-1 and Bif-1, increases tumor susceptibility in mice while the overexpression of these proteins can repress cancer cell growth. However, autophagy is critical for the survival of cancer cells within the nutrient poor and hypoxic core of solid tumors. Cell Meter™ Autophagy Kit employs Autophagy 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 Kit is suitable for fluorescence microscope, fluorescence microplate reader.

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 Blue™ working solution
3. Incubate at 37°C for 15 - 60 minutes
4. Wash the 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 channel
Emission:	DAPI channel
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 Blue™ (Component A) into 10 mL of Stain Buffer (Component B) and mix well to make Autophagy Blue™ working solution. Protect from light.

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

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

1. Culture cells to a density optimal for autophagy induction according to your specific induction protocol (about 1 - 2 x 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 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

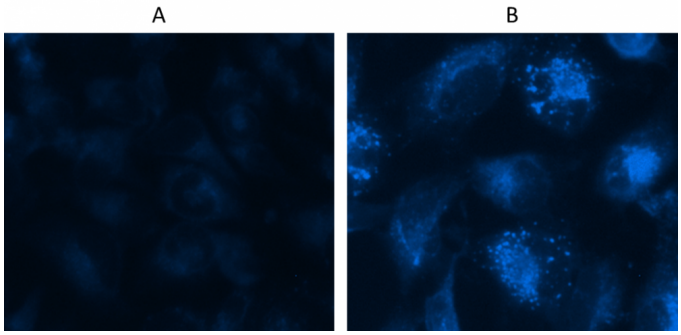

Figure 1. Autophagy Blue™ labeled vesicles are induced by starvation in HeLa cells using Cell Meter™ Autophagy Assay Kit. HeLa cells were incubated in a regular

DMEM medium as control (A) or in a serum-depleted medium as autophagy treatment (B) for 16 hours. Both control cells and starved cells were incubated with Autophagy Blue™ working solution for 30 minutes in a 37 °C, 5% CO_2 incubator, and then washed four times with wash buffer. Cells were imaged immediately under a fluorescence microscope with a DAPI channel. Autophagy is indicated by bright blue dot staining of autophagic vacuoles (B).

DISCLAIMER