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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

Cell Meter™ Autophagy Assay Kit

Blue Fluorescence

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 23000 (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 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 and flow cytometer. Autophagy Blue™ has fluorescence excitation and emission at Ex/Em = 333/518 nm.

Kit Components

Components	Amount
Component A: 500X Autophagy 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 × 10⁴ cells/well → Add Autophagy Blue™ working solution → Incubate at 37°C for 15 min - 1 hour → Wash the cells with wash buffer → Analyze the cells at Ex/Em = 335/520 nm

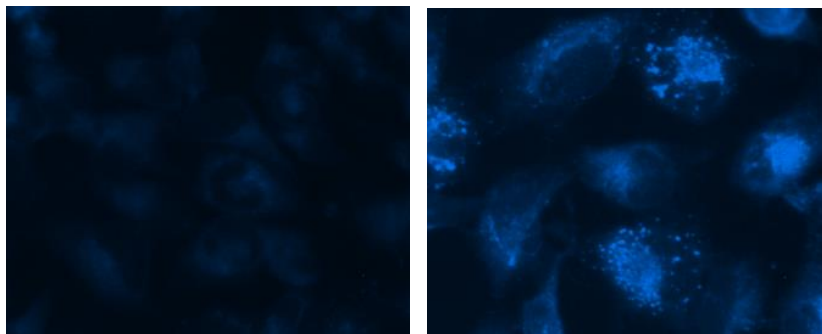
Note: Thaw all the components at room temperature before use.

- Culture cells to a density optimum for autophagy induction according to your specific induction protocol (about 1-2 × 10⁴ 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.
- Prepare Autophagy Blue™ working solution by diluting 20 µL of Autophagy Blue™ (Component A) to 10 mL of Stain Buffer (Component B).
Note: 20 µL of 500 X Autophagy Blue™ (Component A) is enough for one 96-well plate. Aliquot and store unused 500 X Autophagy Blue™ at ≤ -20 °C. Protect from light and avoid repeated freeze-thaw cycles.
- Remove medium, add 100 µL of Autophagy Blue™ working solution (from Step 2) into each well, and incubate the cells in a 37°C, 5% CO₂ 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.
- 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, flow cytometer, or fluorescence microplate reader at Ex/Em = 330/520 nm.

Data Analysis



A

B

Figure 1. Autophagy Blue™ labeled vesicles are induced by starvation in HeLa cells. 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% CO₂ 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).

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.

Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.