3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Autophagy Assay Kit

Catalog Number **MAK138**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
Autophagy is an evolutionarily conserved degradation process that targets long-lived proteins, organelles, and other cytoplasmic components for degradation via the lysosomal pathway. Activation of the autophagy pathway is required for multiple cellular roles, including survival during starvation, the clearance of intracellular components, development, and immunity.

The Autophagy Assay kit provides a simple and direct procedure for measuring autophagy in a variety of cell types using a proprietary fluorescent autophagosome marker (λ_{ex} = 333/λ_{em} = 518 nm). This assay is suitable for fluorescence microscopy and fluorescence microplate readers.

Components
The kit is sufficient for 200 assays.

Autophagosome Detection Reagent, 500× Catalog Number MAK138A	50 µL
Stain Buffer Catalog Number MAK138B	25 mL
Wash Buffer Catalog Number MAK138C	100 mL

Reagents and Equipment Required but Not Provided.
- 96 well flat-bottom plate – If running assays in plates, it is recommended to use black plates with clear bottoms for fluorescence assays.
- Fluorescence multiwell plate reader, fluorescent microscope.

Precautions and Disclaimer
For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The kit is shipped on wet ice. Recommended storage at –20 °C, protected from light.

Procedure
Thaw all reagents and bring to room temperature prior to use.

Sample Preparation
Culture cells in 96 well plate to optimal density for autophagy induction in cells (1–2 × 10^4 cells/well). Also culture a well of cells under same conditions (without induction) to serve as a negative control. Incubate cells for desired time to induce autophagy. The optimal incubation time may need to be determined experimentally. If working with suspension cells, it may be helpful to first coat the plates with poly-D-lysine.

Assay Reaction

1. Following the autophagy induction, prepare a working solution of the Autophagosome Detection Reagent by diluting 20 µL of the 500× solution with 10 mL of the Stain Buffer. This is sufficient volume for one 96 well plate. This volume can be scaled down accordingly if fewer wells will be used. The remaining 500× Autophagosome Detection Reagent can be frozen, protected from light, in single-use aliquots at –20 °C.

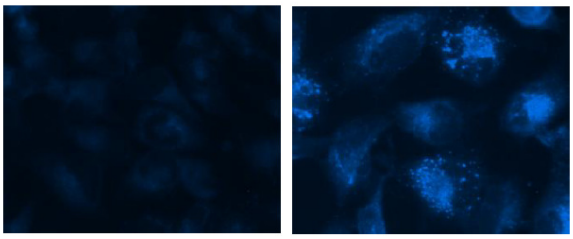
2. Remove the medium from the cells and add 100 µL of the autophagosome detection reagent working solution to each well (samples and controls). If working with suspension cells, spin down cells prior to removing medium and gently resuspend pellet in the autophagosome detection reagent working solution. Incubate the cells at 37 °C with 5% CO_2 for 15 minutes to 1 hour. The appropriate incubation time depends on the individual cell type and cell concentration and should be determined experimentally.

3. Wash the cells with the Wash Buffer 3–4 times by gently adding 100 µL of Wash Buffer to each well. If working with suspension cells, spin down cells and resuspend pellet in wash solution. Remove carefully to prevent dislodging the cells.

4. Measure the fluorescence intensity (λ_{ex} = 360/ λ_{em} = 520 nm) using a fluorescence microscope or microplate reader.
   Note: If the cells do not appear to be sufficiently stained, repeat assay and either increase incubation time or dye concentration.

Results

Figure 1.
Autophagy Assay Kit with 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 the Autophagosome Detection Reagent working solution for 30 minutes at 37 °C with 5% CO_2 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).

SS,LS,MAM 05/20-1