Autophagy Assay Kit ab139484

Product overview
Autophagy Assay Kit ab139484 measures autophagic vacuoles and monitors autophagic flux in live cells using a dye that selectively labels autophagic vacuoles. It can be analyzed using flow cytometry, fluorescent microscopy, or using a microplate reader. The dye has spectral characteristics similar to FITC.

The dye used in the autophagy assay protocol has been optimized by screening dyes for both minimal staining of lysosomes, and bright fluorescence upon incorporation into pre-autophagosomes, autophagosomes, and autolysosomes (autophagolysosomes). The dye is a cationic amphiphilic tracer (CAT) dye that rapidly partitions into cells in a similar manner to drugs that induce phospholipidosis.

This autophagy assay offers a rapid and quantitative approach to monitoring autophagy in live cells without the need for cell transfection.

Chloroquine is included in the kit for use as an inhibitor control. Rapamycin is included in the kit as an autophagy inducer for use as a positive control.

Autophagy assay protocol summary:
- remove growth medium from cells
- add staining mix and incubate for 30 min
- wash cells
- analyze with fluorescence microscopy, flow cytometry, or fluorescent microplate reader

Notes
The reagents provided in this kit are sufficient for 200 flow cytometry tests, 250 fluorescence microscopy test or 3 x 96-well microplate tests.

This product was previously called Autophagy Detection Kit.

**Overview**

<table>
<thead>
<tr>
<th>Product name</th>
<th>Autophagy Assay Kit</th>
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<tbody>
<tr>
<td>Detection method</td>
<td>Fluorescent</td>
</tr>
<tr>
<td>Sample type</td>
<td>Adherent cells, Suspension cells</td>
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<tr>
<td>Assay type</td>
<td>Cell-based</td>
</tr>
<tr>
<td>Assay time</td>
<td>0h 30m</td>
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**Platform**
Microplate reader, Fluor. microscope, Flow cyt.
Storage instructions

Store at -80°C. Please refer to protocols.

<table>
<thead>
<tr>
<th>Components</th>
<th>1 kit</th>
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<tbody>
<tr>
<td>10X Assay Buffer</td>
<td>1 x 30ml</td>
</tr>
<tr>
<td>Autophagy Inducer</td>
<td>1 x 25nmole</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>1 x 7.5µmole</td>
</tr>
<tr>
<td>Green Detection Reagent</td>
<td>1 x 50µl</td>
</tr>
<tr>
<td>Hoechst 33342 Nuclear Stain</td>
<td>1 x 50µl</td>
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Images

Fluorescent microscopy analysis showing nucleus (blue nuclear stain; DAPI filter) and autophagic vesicules (green, FITC filter) in control HepG2 cells or cells treated with 1 uM Rapamycin (ab120224) for 24 hours.

Functional Assay: ab139484 Autophagy Detection Kit
Fluorescent microscopy analysis showing nucleus (blue nuclear stain; DAPI filter) and autophagic vesicles (green, FITC filter) in control HepG2 cells or cells starved in serum free medium for 5 hours to induce the formation of autophagic vesicles. HepG2 cells were also treated with 0.1 mM chloroquine for 24 hours or starved and chloroquine treated for 5 hours (in earlier stages of autophagy, chloroquine induces autophagosome formation).

Jurkat cells (acute T-Cell leukemia), uninduced or treated overnight with 0.5 µM Rapamycin (a typical autophagy inducer) were loaded with Green Detection Reagent, then washed and analyzed by flow cytometry. Results are presented as histogram overlay. Control cells (blue solid line) were stained as well but mostly display low fluorescence. In the samples treated with 500 nM Rapamycin for 18 hours (black solid line), Green dye signal increases about 2-fold, indicating that Rapamycin induced autophagy in Jurkat cells.

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