RAW-Difluo™ mLC3 Cells

Autophagy reporter cells

Catalog code: rawdf-mlc3

https://www.invivogen.com/raw-difluo-mlc3

For research use only

Version 19K14-MM

PRODUCT INFORMATION

Contents

- **1 vial of RAW-Difluo™ mLC3 Cells** (3-7 x 10^6 cells)

IMPORTANT: Cells are shipped frozen. If cells are not frozen upon arrival, contact InvivoGen immediately.

- **1 ml of Zeocin™** (100 mg/ml).

Store Zeocin™ at 4 °C or at -20 °C.*

- **1 ml of Normocin™** (50 mg/ml). Normocin™ is a formulation of three antibiotics active against mycoplasmas, bacteria and fungi.

Store at -20 °C.*

*The expiry date is specified on the product label.

Handling Cells Upon Receipt

Cells must be thawed **immediately** upon receipt and grown according to handling procedures (as described on the next page), to ensure cell viability and proper assay performance.

*Note: **Do not freeze the cells upon receipt** as it may result in irreversible damage to the cell line.*

Disclaimer: We cannot guarantee cell viability if the cells are not thawed immediately upon receipt and grown according to handling procedures.

Cell Line Stability

Genetic instability is a biological phenomenon that occurs in all stably transfected cells. Cells will undergo genotypic changes resulting in reduced responsiveness over time in normal cell culture conditions. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages.

To ensure maximum efficiency, do not passage RAW-Difluo™ mLC3 cells more than 20 times. RAW-Difluo™ mLC3 cells should be maintained in growth medium supplemented with the selection antibiotic Zeocin™ (100 µg/ml).

Quality Control

- RAW-Difluo™ mLC3 cells have been tested for their ability to respond to autophagic inducers.
- The stability of this cell line for 20 passages following thawing has been verified.
- RAW-Difluo™ mLC3 cells are guaranteed mycoplasma-free.

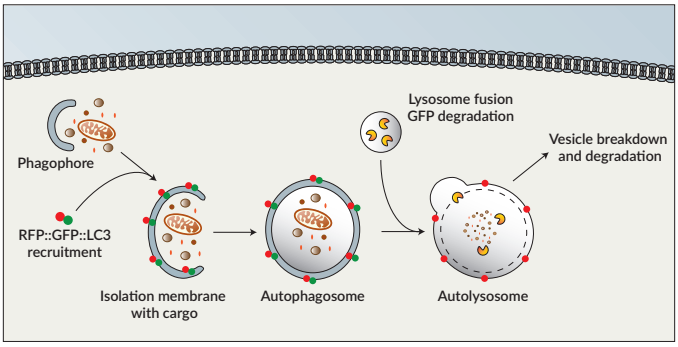
USE RESTRICTIONS

These cells are distributed for research purposes only.

This product is covered by a Limited Use License. By use of this product, the buyer agrees to the terms and conditions of all applicable Limited Use Label Licenses. For non-research use, such as screening, quality control or clinical development, contact info@invivogen.com

BACKGROUND

Autophagy is an essential, homeostatic process by which cytoplasmic materials are degraded in lysosomes. This multi-step process involves isolation of cargo within membranes, autophagosome formation, fusion with lysosomes, degradation and recycling of cargo contents. One key protein used to study this 'autophagic flux' is LC3B (microtubule-associated protein 1 light chain 3). This protein is recruited from the cytosol, matured and bound to the isolation membrane. This localization serves as a marker for autophagic membranes and for monitoring the process as it develops. Chimeric proteins consisting of LC3B fused to a green fluorescent protein (GFP) and a red fluorescent protein (RFP) provide a simple means of monitoring the autophagic process. Autophagosomes marked by RFP::GFP::LC3 show both RFP and GFP signals. After fusion with lysosomes, GFP signal is significantly reduced due to acidic conditions, while RFP signal remains relatively stable.



CELL LINE DESCRIPTION

RAW-Difluo™ mLC3 cells are autophagy reporter cells derived from the murine RAW 264.7 macrophage cell line, a physiologically relevant cell line. They express a fusion protein RFP::GFP::LC3, in which the N-terminus of human LC3B is fused to two fluorescent reporter proteins: a RFP (acid-stable) and a GFP (acid-sensitive). In these cells, the RFP-GFP pair enables monitoring of autophagic flux in real time by detecting the appearance of dual fluorescent red and green RFP::GFP::LC3 puncta or single fluorescent red RFP::LC3 puncta by fluorescence microscopy. Early in autophagy, both RFP and GFP signals are detected. As the fusion of the autophagosomes with the lysosomes progresses, the GFP fluorescence diminishes, leaving only the RFP fluorescence visible. The percentages of RFP-GFP positive and of RFP positive cells can be determined and these values can be used to assess autophagic flux, using methods described previously[1,2]. RAW-Difluo™ mLC3 cells are resistant to Zeocin™.

1. Loos B. *et al.* 2014. Defining and measuring autophagosome flux- concept and reality. Autophagy. 2014;10(11):2087-96. 2. Kimura S. *et al.*, 2007. Dissection of the autophagosome maturation process by a novel reporter protein, tandem fluorescent-tagged LC3. Autophagy. 3(5):452-60.

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FAQ Any questions about our cell lines?
Visit our FAQ page.

InvivoGen
www.invivogen.com

SAFETY CONSIDERATIONS

Biosafety Level 2

HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% heat-inactivated fetal bovine serum (FBS; 30 min at 56 °C), 100 µg/ml Normocin™, Pen-Strep (100 U/ml-100 µg/ml)
- **Freezing Medium:** DMEM, 20% FBS, 10% DMSO
- **Test Medium:** DMEM 4.5 g/l glucose, 2 mM L-glutamine, 10% heat-inactivated FBS, Pen-Strep (100 U/ml-100 µg/ml) **without Normocin™ and Zeocin™**

Required Selective Antibiotic

- Zeocin™

Initial Culture Procedure

The first propagation of cells should be for generating stocks for future use. This ensures the stability and performance of the cells for subsequent experiments.

1. Thaw the vial by gentle agitation in a 37 °C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid.
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol.

Note: All steps from this point should be carried out under strict aseptic conditions.

3. Transfer cells into a larger vial containing 15 ml of pre-warmed growth medium. **Do not add selective antibiotics until the cells have been passaged twice.**
4. Centrifuge vial at 1000-1200 RPM (RCF = 200-300 g) for 5 minutes.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium without selective antibiotics.
6. Transfer the vial contents to a T-25 culture flask containing 5 ml of growth medium.
7. Place the culture at 37 °C in 5% CO_2.

Frozen Stock Preparation

1. Resuspend cells at a density of 5-7 x 10^6 cells/ml in freezing medium freshly prepared with cold DMEM.

Note: A T-75 culture flask typically yields enough cells for preparing 3-4 frozen vials.

2. Transfer 1 ml of cell suspension into cryogenic vials.
3. Place vials in a freezing container and store at -80 °C overnight.
4. Transfer vials to liquid nitrogen for long term storage.

Note: If properly stored, cells should remain stable for years.

Cell Maintenance

1. After cells have recovered, subculture the cells in growth medium with an initial seeding density of 1.5 x 10^4 cells per cm^2 (e.g. ~1 x 10^6 cells in a T-75 culture flask). To maintain selection pressure, add 200 µg/ml of Zeocin™ to the growth medium every other passage.
2. Renew growth medium twice a week.
3. Using a cell scraper, cells should be passaged when a 70-80% confluency is reached. Do not let the cells grow to 100% confluency.

Note: Do **not** *use trypsin.*

Cell-Handling Recommendations

To ensure the best results:

- Use RAW-Difluo™ mLC3 cells with less than 20 passages.
- Handling of cells should be as short as possible to prevent any damage resulting from a prolonged stay at room temperature without 5% CO_2.

Induction of RAW-Difluo™ mLC3 cells

Day 1

1. Prepare a RAW-Difluo™ mLC3 cell suspension at ~125,000 cells/ml in test medium.

Notes:

- Prior to treatment, RAW-Difluo™ mLC3 cells display some RFP::LC3 puncta and rare RFP::GFP::LC3 puncta due to basal autophagy that occurs constitutively during cell propagation.

- It is important that the cells are <80 % confluent to limit basal autophagy.

2. Add 500 µl of cell suspension (~62,500 cells) per well of a 24-well plate.
3. Incubate overnight at 37 °C in a 5% CO_2 incubator.

Day 2

1. Remove test medium and gently rinse cells with pre-warmed, sterile phosphate buffered saline (PBS; pH 7.4).
2. Add 450 µl of test medium to every well of a 24-well plate.
3. Add 50 µl of test compound (autophagy inducer or inhibitor) per well, include a positive control (e.g. rapamycin at a final concentration of 25 µM) and sterile PBS as a negative control.
4. Incubate at 37 °C.
5. Monitor the autophagic flux at different time intervals (e.g. after 30 min, 1h, 2h30 and 6h) using a high-resolution fluorescent microscope with the appropriate optical filters (see Spectral properties for GFP and RFP below).

Notes:

- For better visualization of the autophagic flux, rinse the cells twice to remove the autophagy inducer when autophagy has reached its peak (e.g. after at least 2 h for 25 µM rapamycin)

- At peak autophagy induction, visualization of individual GFP-LC3 and RFP-LC3 puncta can be challenging due to the small size of RAW 264.7 cells. Assessment of puncta can be facilitated by image analysis software.

Optional: To maintain cells at a certain time point in the autophagic flux, we recommend to fix cells using ice-cold methanol:acetone (1:1) for 10 min at 4 °C. Some fluorescent dye leakage may occur. An autophagy inhibitor such as Bafilomycin A can be used to enhance the signal by puncta accumulation.

Fluorescent puncta	Basal level	Autophagosome	Autolysosome
RFP::GFP::LC3	+/-	+++	+/-
RFP::LC3	+	++++	+++

Typical results of autophagic flux

Spectral properties of GFP

Excitation λ max: 480 nm
Emission λ max: 505 nm

Spectral properties of RFP

Excitation λ max: 555 nm
Emission λ max: 584 nm

RELATED PRODUCTS

Product	Description	Cat. Code
Bafilomycin A	Autophagy inhibitor	tlrl-baf1
Rapamycin	Autophagy inducer	tlrl-rap
HeLa-Difluo™ hLC3	Autophagy reporter cells	heldf-hlc3
Wortmannin	Autophagy inhibitor	tlrl-wtm
Zeocin™	Selection antibiotic	ant-zn-1

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