THP1-Difluo™ hLC3 Cells

Autophagy reporter cells

Catalog code: thpdf-hlc3

https://www.invivogen.com/thp1-difluo-hlc3

For research use only

Version 23H22-NJ

PRODUCT INFORMATION

Contents and Storage

- 3-7 x 10^6 of THP1-Difluo™ hLC3 cells in a cryovial or shipping flask. *IMPORTANT: If cells provided in a cryovial are not frozen upon arrival, contact InvivoGen immediately.*
- 1 ml of Zeocin® (100 mg/ml). Store at 4 °C or at -20 °C.*
- 1 ml 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 Frozen Cells Upon Arrival

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

Note: **Avoid freezing 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.

IMPORTANT: For cells that arrive in a shipping flask please refer to the enclosed 'cell recovery procedure'.

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 THP1-Difluo™ hLC3 cells more than 20 times. THP1-Difluo™ hLC3 cells should be maintained in growth medium supplemented with the selection antibiotic Zeocin® (100 µg/ml).

Quality Control

- THP1-Difluo™ hLC3 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.
- THP1-Difluo™ hLC3 cells are guaranteed mycoplasma-free.

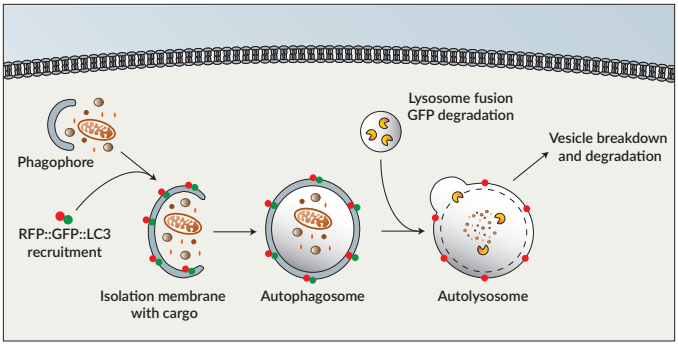
USE RESTRICTIONS

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

THP1-Difluo™ hLC3 cells are autophagy reporter cells derived from the THP-1 human monocytic 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: RFP (acid-stable) and 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].

THP1-Difluo™ hLC3 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.

SAFETY CONSIDERATIONS

Biosafety Level 1

HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** RPMI 1640, 2 mM L-glutamine, 25 mM HEPES, 10% heat-inactivated fetal bovine serum (FBS; 30 min at 56°C), 100 µg/ml Normocin™, Pen-Strep (100 U/ml-100 µg/ml)

Initial culture of all THP-1 derived cells must be performed in growth medium containing 20% heat-inactivated FBS.

Note: The use of Normocin™ together with Pen-Strep is required to keep the cells free of microbial contaminants.

- **Test Medium:** RPMI 1640, 2 mM L-glutamine, 25 mM HEPES, 10% heat-inactivated FBS, Pen-Strep (100 U/ml-100 µg/ml)
- **Freezing Medium:** 95% (v/v) FBS and 5% (v/v) DMSO

Required Selection 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% (v/v) 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 (with 20% heat-inactivated FBS). **Do not add selective antibiotics until the cells have been passaged twice.**
4. Centrifuge vial at 150 x g (RCF) for 10 minutes.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium (with 20% heat-inactivated FBS).
6. Transfer the vial contents to a 25 cm^2 tissue culture flask containing 5 ml of growth medium (with 20% heat-inactivated FBS).
7. Place the flask containing cells 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 freshly prepared freezing medium.

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

2. Dispense 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 (after at least two passages), subculture the cells in growth medium (with 10% heat-inactivated FBS). To maintain selection pressure, add 100 µg/ml of Zeocin® to the growth medium every other passage.
2. Pass the cells every 3 days by inoculating 5 x 10^5 cells/ml. Do not allow the cell concentration to exceed 2 x 10^6 cells/ml.

Note: Handling of cells should be as short as possible to prevent any damage resulting from the prolonged stay at room temperature without 5% CO_2.

Induction of THP1-Difluo™ hLC3 cells

Day 1

1. Prepare a cell suspension at 8 x 10^5 cells/ml in test medium.
2. For better microscopy visualization, we recommend differentiation of THP-1 monocytes into adherent macrophages with PMA. In a 24-wellplate, distribute 475 µl of the THP1-Difluo™ hLC3 cell suspension and treat cells with 25 µl of PMA at 1 µg/ml (50 ng/ml final concentration) for 3 hours at 37°C in 5% CO_2.
3. Gently remove medium and rinse cells twice with pre-warmed, sterile phosphate buffered saline (PBS; pH 7.4). Add 500 µl of RPMI.

Notes:

*- Test medium does **not** contain antibiotics (Normocin™ or Zeocin®).*

- Prior to treatment, THP1-Difluo™ hLC3 cells display some RFP::LC3 puncta and rare RFP::GFP::LC3 puncta due to basal autophagy that occurs constitutively during cell propagation.

Day 4

1. Remove test medium and gently rinse cells with pre-warmed, sterile PBS.
2. Add 450 µl of test medium to every well of a 24-well plate.
3. Add 50 µl of test compound (autophagy inducers or inhibitors) 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 4 h, 8 h, 16 h and 24 h) 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).

- To enable the measurement of the average size and number of puncta per cell, image analysis software can be used if desired.

Optional: For convenience and kinetics studies, cell samples may be fixed 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.

Typical results of autophagic flux

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

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
PMA	Phorbol myristate acetate	tlrl-pma
Rapamycin	Autophagy inducer	tlrl-rap
Zeocin®	Selection antibiotic	ant-zn-1