Premo™ Autophagy Sensors (LC3B-FP) *BacMam 2.0*

Catalog nos. P36235, P36236

Table 1. Contents and storage information.

Material	P36235	P36236	Concentration	Storage*
LC3B-FP (Component A)	1 mL of LC3B-GFP	1 mL of LC3B-RFP	~1 × 10^8 viral particles/mL	• 2–6°C • Protect from light • DO NOT FREEZE
Control LC3B(G120A)-FP (Component B)	200 µL of LC3B(G120A)-GFP	200 µL of LC3B(G120A)-RFP	~1 × 10^8 viral particles/mL	
Chloroquine diphosphate (Component C)	1 mL	1 mL	30 mM aqueous solution	• ≤25°C • Protect from light
Approximate fluorescence excitation/emission maxima: GFP: 485/520 in nm; RFP: 555/584 in nm.				
***Stability:** When stored as directed, this kit is stable for at least 6 months.				

Introduction

Autophagy describes the segregation and delivery of cytoplasmic cargo, including proteins and organelles, for degradation by hydrolytic enzymes in "autophagolysosomes", also referred to as "autolysosomes". Although first described in 1963, it has only been in the past decade that this pathway has been the subject of intense research to gain further insight into the role basal autophagy plays in cell homeostasis and development. Efforts are also directed to further elucidate the role of induced autophagy as a cell survival response to stress, microbial infection, and disease (e.g., neurodegeneration, cancer).[1–3]

The LC3B protein plays a critical role in autophagy. Normally, this protein resides in the cytosol, but following cleavage and lipidation with phosphatidylethanolamine, LC3B associates with the phagophore. This localization can be used as a general marker for autophagic membranes (Figure 1).

The Premo™ Autophagy Sensor combines the selectivity of an LC3B-fluorescent protein (FP) chimera with the transduction efficiency of the BacMam 2.0 technology. BacMam reagents (insect **Bac**ulovirus with a **Mam**malian promoter) are safe to handle (Biosafety Level 1) because they are non-replicating in mammalian cells. They are also non-cytotoxic and ready-to-use. Unlike expression vectors, BacMam reagents enable titratable and reproducible expression and offer high co-transduction efficiency. Multiple BacMam reagents can therefore be readily used in the same cell. Recent improvements made to the BacMam system, BacMam 2.0, enable efficient transduction in a wider variety of cells, including neurons and neural stem cells (NSCs). The two-step protocol for imaging autophagy involves simply adding the BacMam LC3B-FP to the cells and incubating them overnight for protein expression.

Each Premo™ Autophagy Sensor Kit includes the BacMam LC3B-FP, a control BacMam LC3B(G120A)-FP, and chloroquine diphosphate to induce autophagosomes (Figure 2). The mutation on the control BacMam LC3B prevents its cleavage and subsequent lipidation during normal autophagy, and protein localization remains cytosolic and diffuse. Following treatment with chloroquine diphosphate, normal autophagic flux is disrupted and autophagosomes accumulate as a result of the increased lysosomal pH that inhibits lysosomal fusion with the autophagosomes.

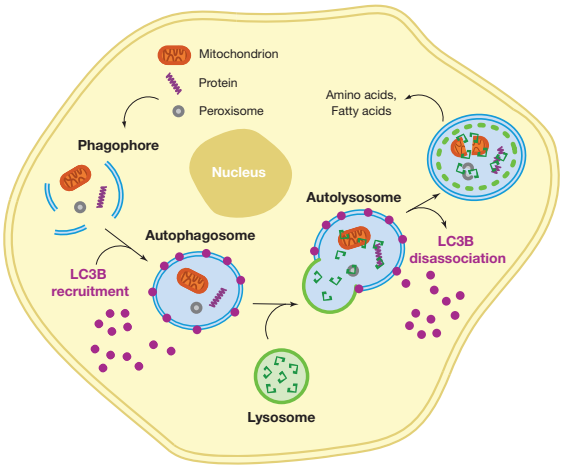

Figure 1. Schematic depiction of the autophagy pathway in a eukaryotic cell. The first step involves the formation and elongation of the isolation membranes or phagophore. The second step entails the expansion and sequestering of the cytoplasm and formation of the double-membrane autophagosome and includes the association of the cytosolic LC3 protein. Fusion of lysosomes with autophagosome to generate the autolysosome is the penultimate step. In the fourth and final phase, the cargo is degraded.

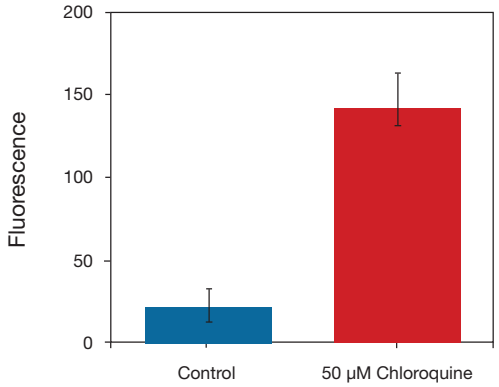

Figure 2. Autophagy detection with Premo™ Autophagy Sensor. HeLa cells were plated at 5,000 cells per well and allowed to adhere overnight. Cells were then transduced with LC3B-GFP. 24 hours later, the cells were incubated with 50 µM chloroquine or left untreated (control) for 16 hours. Analysis was performed by quantifying the fluorescence from vesicular structures in the perinuclear region using the Thermo Scientific Cellomics® ArrayScan® VTI platform with Compartmental Analysis.

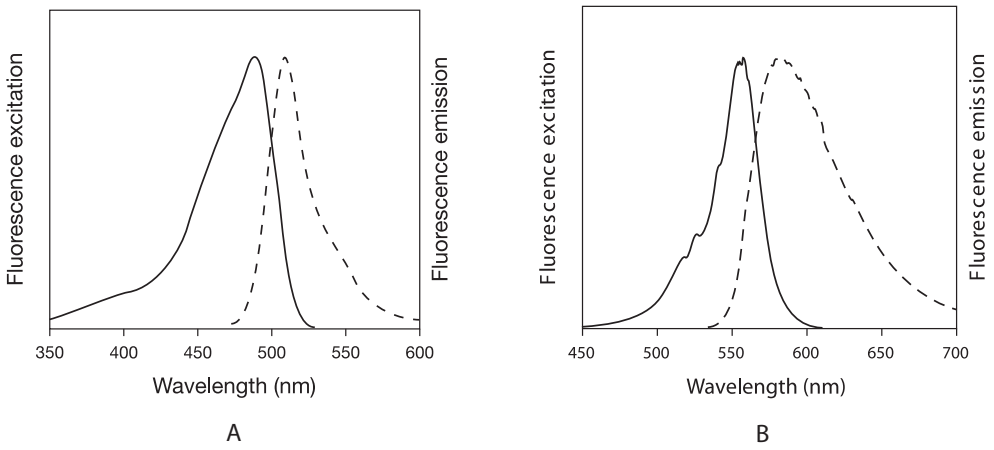

Figure 3. Fluorescence excitation and emission spectra for Premo™ LC3B-Green Fluorescent Protein (LC3B-GFP, panel A) and Premo™ LC3B-Red Fluorescent Protein (LC3B-RFP, panel B).

Before Starting

Materials Required but Not Provided

- Cell culture medium

Guidelines for Working with BacMam Reagents

- The following protocol is based on a 2 mL labeling volume and ~40,000 cells plated in a 35-mm dish or 1 well of a 6-well culture plate, and an MOI (multiplicity of infection) of 30 plaque forming units (pfu) per cell.
- For applications that require a larger number of cells, such as high-content screening (HCS), we recommend transducing the cells in a 10-cm dish or a T-75 flask and increasing the labeling volume to 10 mL with a proportionate increase in the volume of the virus. Following an overnight incubation under normal growth conditions, trypsinize and count cells for distribution to appropriate plates at cell number desired.
- For optimal results you may alter the MOI, cell density, temperature, and incubation time.
- We recommend transducing cells at a confluency of about 70% for best results.
- For first time users of BacMam reagents, we recommend exceptionally well-transduced cells like U-2 OS (ATCC CCL-. 11226).

Experimental Protocols

Protocol The following protocol was optimized using cells that have been plated. You can also treat the cells in suspension prior to plating.

1.1 Plate the cells at the desired density and allow them sufficient time to adhere. BacMam reagents work best when used on low-passage-number cells.

1.2 Calculate the volume of LC3B-FP (Component A) and LC3B(G120A)-FP (Component B) using the equation below:

$$\text{mL of LC3B-FP or LC3B(G120A)-FP} = \frac{\text{(number of cells) (MOI)}}{(1 \times 10^8)}$$

where the number of cells is the estimated total number of cells at the time of cell labeling, MOI (multiplicity of infection) is the number of viral particles per cell, and 1×10^8 is the number of viral particles per mL of the reagent.

For example, to label 40,000 cells with an MOI of 30,

$$\text{mL of LC3B-FP or LC3B(G120A)-FP} = \frac{(40{,}000)\ (30)}{(1 \times 10^8)} = 0.012 \text{ mL } (12\ \mu\text{L})$$

1.3 Mix each LC3B reagent by inversion to ensure a homogenous solution.

1.4 Add the LC3B reagent directly to the cells in complete cell medium and mix gently.

1.5 Incubate the cells overnight (≥16 hours) for LC3B expression.

1.6 *Optional*: Treat the control cells with 30–100 µM chloroquine (Component B) for 12–16 hours.

1.7 Image and analyze the cells using the appropriate instrument filter sets. Refer to Figure 3 for spectral characteristics of GFP and RFP. Autophagosomes are typically located in the perinuclear region.

Note: Premo™ Autophagy Sensors were designed for use in live-cell imaging of autophagy. The cell-permeant nucleic acid stains Hoechst 33342 and HCS NuclearMask™ Blue stains are spectrally compatible with the Premo™ Autophagy Sensor fluorescence. Should you prefer fixed cell analysis, the fluorescence from GFP and RFP has been demonstrated to be resistant to fixation with 4% formaldehyde and permeabilization with 0.1% Triton® X-100. Fixation and permeabilization enables processing of labeled cells with antibodies to other cellular targets. We recommend fixed cell format for large sample sizes, such as for HCS.

1.8 Image the cells using the appropriate filters for GFP or RFP (see Figure 3).

Note: Overexpression of LC3B has been shown to promote aggregation of this protein in a non-autophagic dependent manner;[4] for this reason, we recommend titrating the expression levels by varying the MOI.

References

1. Molecular Cell Biology 8, 931 (2007); **2.** Genes & Development 21, 2861 (2007); **3.** Drug Discovery 6, 304 (2007); **4.** Meth Enz 452, 25 (2009).

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
P36235	Premo™ Autophagy Sensor LC3B-GFP *BacMam 2.0*	1 kit
P36236	Premo™ Autophagy Sensor LC3B-RFP *BacMam 2.0*	1 kit
Related Products		
C10596	CellLight™ Lysosomes-GFP *BacMam 2.0*	1 mL
C10597	CellLight™ Lysosomes-RFP *BacMam 2.0*	1 mL
C10600	CellLight™ Mitochondria-GFP *BacMam 2.0*	1 mL
C10601	CellLight™ Mitochondria-RFP *BacMam 2.0*	1 mL
H3570	Hoechst 33342, trihydrochloride, trihydrate *10 mg/mL solution in water*	10 mL
H10325	HCS NuclearMask™ Blue	65 µL
L7528	LysoTracker® Red DND-99 *1 mM solution in DMSO* *special packaging*	10 × 50 µL
L7535	LysoTracker® Red DND-26 *1 mM solution in DMSO* *special packaging*	10 × 50 µL
L10382	LC3B Antibody Kit for Autophagy *rabbit polyclonal LC3B* *includes autophagosome inducer*	1 kit
M7510	MitoTracker® Orange CMTMRos *special packaging*	10 × 50 µL
M7512	MitoTracker® Red CMXRos *special packaging*	10 × 50 µL
M7514	MitoTracker® Green FM *special packaging*	10 × 50 µL
M22423	MitoTracker® Deep Red *special packaging*	10 × 50 µL

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