User Manual

# Lv-LC3 Autophagy Detection

## Table of Contents

## Safe Use of Lentivirus (Lv)

1. Lentivirus (Lv) related experiments should be conducted in biosafety level 2 facilities (BL-2 level).

2. Please equip with lab coat, mask, gloves completely, and try your best to avoid exposing hand and arm.

3. Be careful of splashing virus suspension. If biosafety cabinet is contaminated with virus during operation, scrub the table-board with solution comprising 70% alcohol and 1% SDS immediately. All tips, tubes, culture plates, medium contacting virus must be soaked in chlorine-containing disinfectant before disposal.

4. If centrifuging is required, a centrifuge tube should be tightly sealed. Seal the tube with parafilm before centrifuging if condition allowed.

5. Lentivirus related animal experiments should also be conducted in BL-2 level.

6. Lentivirus associated waste materials need to be specially collected and autoclaved before disposal.

7. Wash hands with sanitizer after experiment.

## Storage and Dilution of Lentivirus

### Storage of Lentivirus

Virus can be stored at 4°C for a short time (less than a week) before using after reception. Since Lentiviruses are sensitive to freeze-thawing and the titer drops with repeated freeze-thawing, aliquot viral stock should be stored at -80°C freezer immediately upon arrival for long-term usage. While virus titer redetection is suggested before using if the lentiviruses have been stored for more than 12 months.

### Dilution of Lentivirus

Dissolve virus in ice water if virus dilution is required. After dissolving, mix the virus with medium, sterile PBS or normal saline solution, keeping at 4°C (using within a week).

### *Precautions*

- Avoid lentivirus exposure to environmental extremes (pH, chelating agents like EDTA, temperature, organic solvents, protein denaturants, strong detergents, etc.)

- Avoid introducing air into the lentivirus samples during vortexing, blowing bubbles or similar operations, which may result in protein denaturation.

- Avoid repeated freezing and thawing.

- Avoid exposing to “regular” plastics (especially polystyrene or hydrophobic plastics) for prolonged periods in liquid phase. Most lentivirus viruses are very sticky and loss can occur if exposed to regular plastics, including tubes, cell culture plates, pipette tips, if not frozen. It is best to store lentivirus in siliconized or low protein binding tubes. Pluronic F-68 used at 0.01%-0.1% in the formulation buffer will minimize sticking if regular plastics are used.

• Avoid diluting lentivirus into low salt solution. Some lentiviruses aggregate in low salt solution, which will be non-infectious.

## Introduction of Lentivirus

**Recombinant Lentivirus (rLv)**

Lentivirus (lente-, Latin for "slow") is a genus of retroviruses, causing chronic and deadly diseases by long incubation periods in human or other mammalian species [1]. To date, 5 serogroups have been recognized according to the vertebrate hosts they are associated with (primates, sheep and goats, horses, domestic cats, and cattle). Among them, the primate lentiviruses are distinguished by the utilization of CD4 surface protein as a receptor and the absence of dUTPase [2]. Derived from HIV-1, lentiviruses can integrate a significant amount of viral cDNA into the host genome, mediate stable and long-term transgene expression, and efficiently infect dividing cells and nondividing cells, which makes lentivirus an attractive gene delivery vehicle in most cell types [3].

Considering the key safety concerns during the use of HIV-derived lentivirus vectors, recombinant lentivirus has been designed and widely used for gene delivery in most cell types. As a research tool used to introduce a gene product into *in vitro* systems or animal models, lentiviral vector has been put into large-scale efforts to down-regulate or up-regulate gene expression in high-throughput formats, allowing researchers to examine the necessity and effects of transgenes in disease model systems, which is an indispensable for the discovery of novel transgenic drugs.

Nowadays, several generations of lentivirus packaging system are developed, in which the second-generation lentivirus vector and the third-generation lentivirus vector are the two most popular ones. The current method of the recombinant lentivirus production in Genemedi is based on three plasmids co-transfection system, involving the co-transfection of 3 plasmids (lentivirus series plasmid containing gene of interest (GOI) pLv-GOI, envelope expressingplasmid pMD2G and packaging plasmid pSPAX2) into 293T cells to generate lentivirus vectors.

**Autophagy**

Autophagy also known as type II cell-death, defines an evolutionarily conserved process of recycling, whereby damaged organelles and macromolecular substances are broken down into their constituent parts within the lysosomes, which is tightly regulated by the autophagy related gene (Atg). Three kinds of autophagy have been described to date: macroautophagy, microautophagy and chaperone-mediated autophagy.

Macroautophagy, also referred to as 'autophagy' in general, which consists of three main steps: 1) Induction and phagophore formation; 2) Phagophore elongation and autophagosome formation; 3) Fusion, degradation and recycling. Members of the LC3 family play a key role in the maturation of the autophagosome. LC3 precursors, diffusely distributed in the cytosol, are proteolytically processed to form LC3-I. Upon initiation of autophagy, C-terminal glycine of LC3-I is modified by addition of a phosphatidylethanolamine to form LC3-II, which translocates rapidly to nascent autophagosomes in a punctate distribution.

Microautophagy, mediated by direct lysosomal engulfment of the cytoplasmic cargo.

Chaperone-mediated autophagy (CMA), refers to the chaperone-dependent selection of soluble cytosolic proteins that are then targeted to lysosomes and directly translocated across the lysosome membrane for degradation.

**Lv-mRFP-GFP-LC3 Biosensor**

For autophagy study, Genemedi supply autophagy biosensor, in which GFP and/or RFP tags are fused at the C-termini of the autophagosome marker LC3, allowing to detect the intensity of autophagy flux in real-time with more accuracy, clarity and intuitiveness. These biosensors provide an enhanced dissection of the maturation of the autophagosome to the autolysosome, which capitalizes on the pH difference between the acidic autolysosome and the neutral autophagosome. The acid-sensitive GFP will be degraded in autolysosome whereas the acid-insensitive RFP will not. Therefore, the change from autophagosome to autolysosome can be visualized by imaging the specific loss of the GFP fluorescence, leaving only red fluorescence.

Taking advantage of RFP-GFP-LC3 and GFP-LC3 labeling system, Genemedi has launched the production service of Lv-RFP-GFP-LC3 and Lv-GFP-LC3, which can be used to observe autophagy flux and monitor the intensity of autophagy flux in real-time *in vivo* or *in vitro*.

## Overall Experiment Procedure of Lentivirus Production

The Lv-LC3 Autophagy Flux biosensor virus can be packaged using 293T cells, purified with CsCl density gradient centrifugation method, and titer is detected by plaque assay. The detailed protocol can be consulted in the Lentivirus User Manual on the Genemedi website.

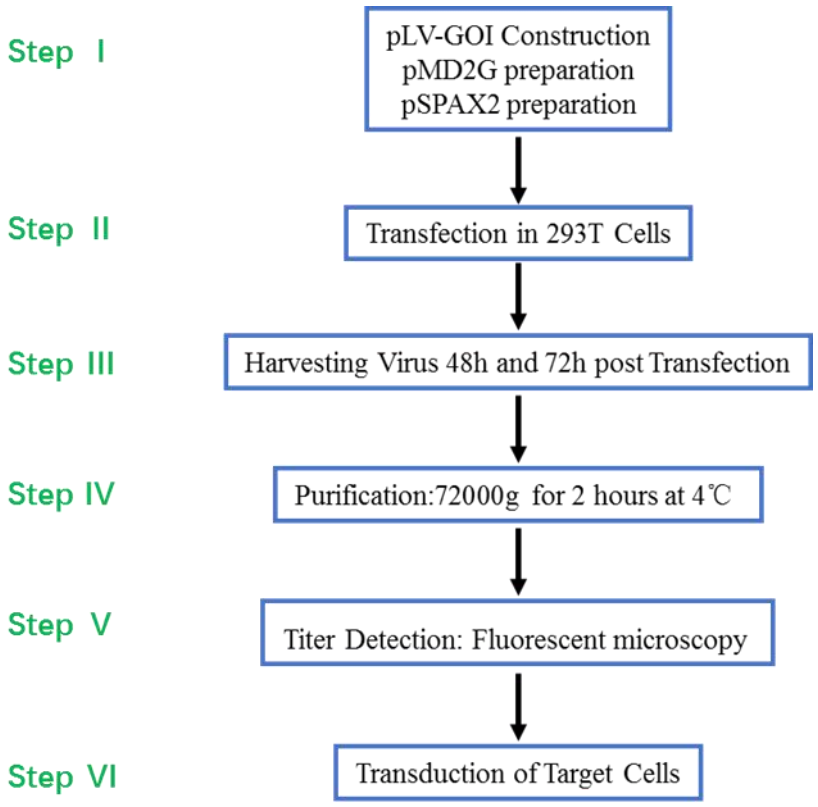


**Figure 1. Lentivirus packaging experiment flow chart.**

## Lv-LC3 Autophagy Flux Biosensor Virus Transduction *in vitro*

After virus titer detection, the Lv-LC3 autophagy flux biosensor can be tested *in vitro*. The detailed recommended protocol for *in vitro* cell transduction can be consulted from Lentivirus User Manual. Infect primary cells, such as neuronal cells, with Lentivirus-LC3 autophagy flux biosensor virus at confluency about 70%-80%. 24h post infection, change the medium. 96h post infection, perform live cell imaging with confocal microscopy and data analysis with ImageJ software.

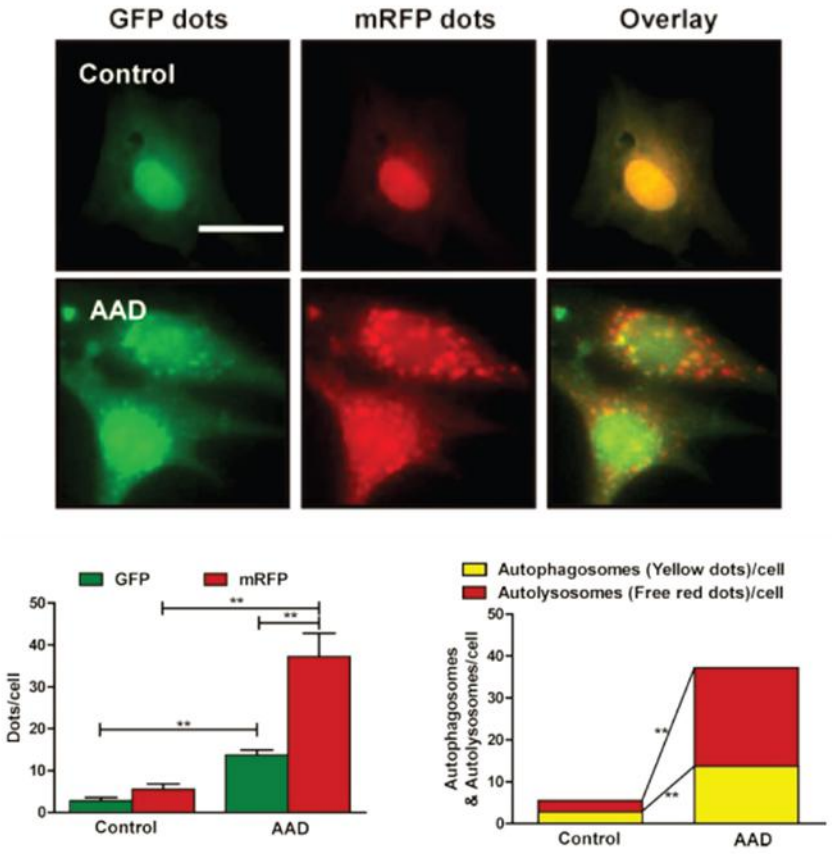


**Figure 2. Confocal microscopic analysis of autophagosomes after amino acids deprivation (AAD).**

***Notes for infection of special cell lines.***

**1. <u>Suspension cells</u>**

We recommend using flat fillet centrifuging transfection to infect suspension cells or semi-suspension cells. Add virus suspension into cell culture dish, sealing tightly, and centrifuge at low speed of 200g for 1 hour in the flat fillet centrifuge. Place cells in cell culture incubator after centrifuging transfection. If the flat fillet centrifuge is inaccessible, you can suspend the cells and transfer cells into centrifuge tubes, followed by low-speed centrifuge, and discard the most of supernatant. Add virus suspension into the tubes, resuspending cells, place it at room temperature for 15 min (no more than 30 min), and transfer the cells and virus suspension into plate to culture. Replace with fresh culture medium the next day.

**2. Cells difficult to infect**

For cells difficult to infect, like DC cells, we recommend repeated infections. Replace with fresh virus suspension 24 hours after the first infection. Repeated infections can increase the infection efficiency markedly.

**3. Non-dividing primary cells**

We recommend high-titer adenovirus to infect these cells like BMSC.

## References


1. Salgado CD and JM Kilby. (2009). Retroviruses and other latent viruses: the deadliest of pathogens are not necessarily the best candidates for bioterrorism. J S C Med Assoc 105:104-6.

2. Piguet V, O Schwartz, S Le Gall and D Trono. (1999). The downregulation of CD4 and MHC-I by primate lentiviruses: a paradigm for the modulation of cell surface receptors. Immunol Rev 168:51-63.

3. Cockrell AS and T Kafri. (2007). Gene delivery by lentivirus vectors. Mol Biotechnol 36:184-204.


## Contact Information


Genemedi Biotech. Inc.

For more information about lentivirus, please visit: www.genemedi.net/i/lentivirus-packaging
For more information about Genemedi products and to download manuals in PDF format, please visit our web site: www.genemedi.net
For additional information or technical assistance, please call or email us

Worldwide：+86-21-50478399
Fax：+86-21-50478399
E-mail: support@genemedi.net




GENEMEDI
Innovation In Gene Therapy