User Manual

AdV-LC3 Autophagy Detection

Table of Contents

Safe Use of Adenovirus (AdV)

1. AdV 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. AdV related animal experiments should also be conducted in BL-2 level.

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

7. Wash hands with sanitizer after experiment.

Storage and Dilution of AdV

Storage of AdV

Virus can be stored at 4°C for a short time (less than a week) before using after reception. Since AdV viruses 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 AdV viruses have been stored for more than 12 months.

Note: 1. Repeated freeze-thaw cycles must be avoided in case of a downside effect on virus titer (for each freeze-thaw cycle, there would be a 10%-50% decrease). Genemedi will provide rAdV products in small aliquots (200 μl/tube) that can be directly stored in -80 centigrade for multiple usage.
2. For viruses stored more than 6 months, it is suggestive to re-analyze virus titer before use.

Dilution of AdV

To properly thaw rAdV frozen aliquots, transfer viruses from -80 ℃ freezer to an ice-water bath till completely melted. When melted, add proper amount of sterile PBS or serum-free culture medium, and keep in 4℃ for no more than a week.

Precautions

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

- Avoid introducing air into the AdV 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 AdV 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 AdV 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 AdV into low salt solution. Some viruses aggregate in low salt solution, which will be non-infectious.

Introduction

Recombinant Adenovirus (rAdV)

Recombinant adenovirus (rAdV) is a replication-defective adenoviral vector, which is widely used for a variety of purposes including gene transfer and engineering, vaccination and gene therapy1,2. There are several advantages of using rAdV as a gene transfer mediator. Firstly, it can deliver as large as 8 kilo-base (kb) gene sequences into cells and tissues without insertion of exogenous fragment in the genome. Secondly, almost all the dividing and non-dividing cells, primary cells and organ tissues can be transduced by rAdV. Moreover, rAdV is easy to operate and expand into large-scale, and the efficiency can reach up to 100%. Thus, rAdV plays an important role in gene engineering research and potential therapeutic treatment of diseases.

The most commonly used adenovirus is serotype 5 (Ad5) of Homo Sapiens consisting of a double-stranded linear DNA molecule at about 36 kb in size1. The cytoplasmic membrane receptors and fibers facilitate endocytosis of adenovirus into cell cytoplasm, where virus particles further migrate into cell nucleus for self-replication using replication machinery of the host3. Once replicated, the virus genome is assembled into its protein shell and released from cells, causing cell lysis3.

Nowadays, several packaging systems of rAdV are developed, in which AdEasy1 and AdMAX2 are the two most popular ones, sharing a common strategy that target gene sequence is cloned into a shuttle vector, then recombined into a viral backbone vector. Early viral transcription units, E1 and E3, are defected in both of these two systems, while E3 gene is not necessary for virus replication1. Thus, packaging of rAdV is usually conducted in cell lines expressing E1 gene, such as HEK-293, HEK-293A etc.2.

In comparison with AdEasy, AdMAX system is relatively easy to handle and can achieve higher virus titer during virus production. This rAdV protocol is developed according to AdMAX system, using a two-vector system composed of a pAd shuttle vector and a rAdV backbone vector pBHGlox(delta) E1-3cre.

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.

AdV-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 AdV-RFP-GFP-LC3 and AdV-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 AdV Production

The AdV-LC3 Autophagy Flux biosensor virus can be packaged using 293A cells purified with ultracentrifugation method, and titer is detected with fluorescent microscopy. The detailed protocol can be consulted in the Lentivirus User Manual on the Genemedi website.

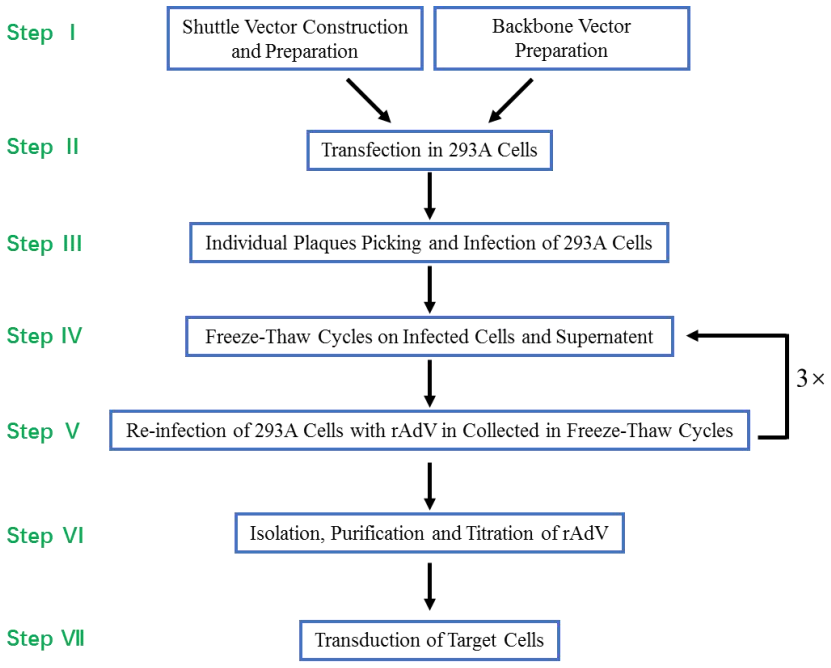

Figure 1. AdV packaging experiment flow chart.

AdV-LC3 Autophagy Flux Biosensor Virus Transduction *in vitro*

After virus titer detection, the AdV-LC3 autophagy flux biosensor can be tested *in vitro*. The detailed recommended protocol for *in vitro* cell transduction can be consulted from AdV User Manual. Infect primary cells, such as neuronal cells, with AdV-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.

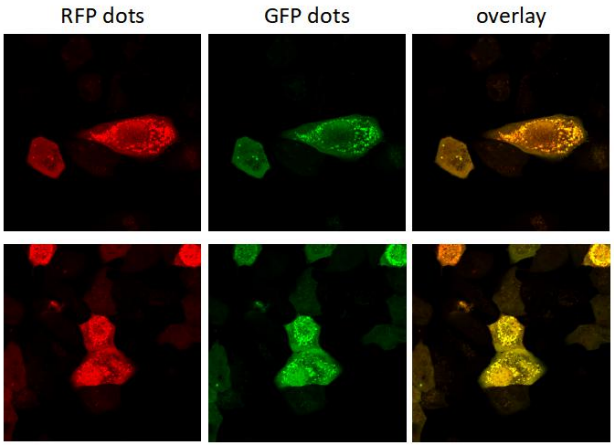

Figure 2. AdV-mRFP-GFP-LC3 indicates vector autophagy flux in Hela cells.

AdV-LC3 Autophagy Flux biosensor virus Transduction *in vivo*

Caution: Purification of rAdV is required for animal injection.

The successful validated AdV-LC3 autophagy flux biosensor virus can be delivered *in vivo*. Genemedi has systematically organized the corresponding gene delivery method and injection volume for mouse and rat tissue infection, please consult our technical support.

About 3 weeks post infection, get the target tissues or organs from animals, embed with OCT, and carry out frozen section using a cryostat. Then fix the slices from frozen section with 4% paraformaldehyde and perform immunohistochemical staining. The autophagy flux can be detected with confocal microscopy and quantitated with the ImageJ software.

References

References

1. Luo J. et al. A protocol for rapid generation of recombinant adenoviruses using AdEasy system. Nat. Protocols. 2 (5), 1236-1247 (2007).
2. He T. C. et al. A simplified system for generating recombinant adenoviruses. PNAS. 95, 2509-2514 (1998).
3. Meier O. & Greber U. F. Adenovirus endocytosis. J. Gene Med. 6 (Suppl 1), S152-S163 (2004).

Contact Information

Genemedi Biotech. Inc.

For more information about adenovirus, please visit: www.genemedi.net/i/adenovirus-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