
General Guidelines for Adenoviral Vector Amplification and Adenovirus Infection of Target Cells

Adenoviral Vector Amplification

Most adenoviral vectors are supplied as a 250ul seed stock. With this stock, customers can produce as much virus stock as they desire. Because of this feature, it is more economical in the long term to use adenoviral vectors instead of transfection reagents for gene transduction, as repeated purchasing of transfection reagents are not required. In addition, adenoviral vectors provide close to 100% gene transduction efficiency in most cell lines tested.

To amplify your adenoviral vector, you will need to grow up 293 cells, which can support the replication of the vectors. Depending on the required viral amount, you will need to grow up different volumes of 293 cells. For example, if 50ml viral supernatant is needed, five 10cm plates should be prepared. To achieve this, we recommend customers to start growing 293 cells in one well of a 6-well plate and in one 10cm dish.

When cells are approximately 60-70% confluent in the 6-well plate, add 100ul of adenoviral vector seed stock to 0.5ml of complete culture medium. Aspirate the culture medium from the well in the 6-well plate and then add the diluted virus onto the 293 cells slowly without dislodging the cells. Return the plate to the 37°C 5% CO₂ incubator for 1-2 hours before adding another 1.5ml of complete culture medium into the well. It will take 4-6 days to see over 95% of the 293 cells are detached from the well; this scenario is called complete CPE (cytopathic effect).

When over 95% of the cells are detached, collect both the medium and cells from the well into a 15ml culture tube. Store the virus at 4°C if you are going to use it for another the next round of amplification (see page 2) within a couple of weeks. Store the virus at -70°C if you are not planning to do further amplification for a while.

Adenoviral vectors are more stable at 4°C and room temperature than lentiviral and retroviral vectors. After repeated testing, we found there is no significant loss of titer when stored at 4°C or room temperature for up to 72 hours. Virus will still be viable after one year of storage at 4°C but long-term storage at -70°C is needed to minimize the stock titer loss. In addition, the sample should be prepared to 5% glycerol for long-term storage at -70°C.

This product is distributed for laboratory use only.

CAUTION: Not for clinical use. The safety and efficacy of this product in clinical uses has not been established.

While adenoviral vectors are being replicated in the 6-well plate, sub-culture the 10cm dish to five 10cm dishes. When 293 cells reach 70% confluence in 10cm dishes, add 300-400ul of crude viral stock from the previous 6-well plate directly into the 10cm culture dishes. It will take another 4-5 days before the completion of CPE. Collect all cells and culture medium into a 50ml culture tube. Freeze once at -70°C and thaw once at 37°C to release the viral particles from cells. Pellet the cell debris by centrifugation at 2,000g for 10 minutes.

One of the significant advantages of adenoviral vector is that it can reach relative high titer even from crude supernatant. Based on our 20 years of viral experience, we have found that adenoviral vector titers were within the following ranges from different preparations:

<u>Viral Titer</u>	<u>Sample Preparation</u>
10^6	Original culture supernatant
10^7	Original culture (medium and cells) after freeze/thaw cycles (up to 3 cycles)
10^8 - 10^{10}	Purification by filter-based methods
10^{10} - 10^{12}	Purification by CsCl banding

Significant lower titers will be expected if your gene of interest is toxic to the packaging 293 cells.

Infection of Target Cells with Adenoviral Vectors

If the viruses are to be used in *in-vitro* cell cultures, virus purification is not required because the viral supernatant will provide 100% gene transduction efficiency in most human cell lines. For *in-vivo* studies (i.e. animal studies), purification is essential to remove defective particles, cell debris, and small amounts of media components, as these contaminants can induce significant immune responses in the host. High-titer adenovirus purification can be achieved by either filter-based or CsCl ultracentrifugation methods.

To infect target cells *in-vitro*, we recommend that 10 to 100 pfu (plaque forming units or infectious viral particles) will be needed for 100% transduction efficiency, depending on the cell types in study. In general, we found that it is convenient to infect target cells in 6-well plates or 10cm culture dishes for preliminary study without precise titer assessment. When target cells are prepared at 70% confluency, aspirate the culture medium from the plate. Infect the cells with enough viral culture supernatant (1ml for 6 well plate and 4-5ml for 10cm dishes) for one hour in a 37°C 5% CO_2 incubator. After the hour of incubation, remove the medium containing the virus and replace it with equal volume of fresh complete culture medium. After 48 or 72 hours, gene transduction can be evaluated by different assays, such as microscope observation if there is a color-giving reporter gene, Western blot or qPCR analysis.

This product is distributed for laboratory use only.

CAUTION: Not for clinical use. The safety and efficacy of this product in clinical uses has not been established.

Safety

Most adenoviral vectors are generated to be replication-defective in target cells. However, it is highly recommended that manipulations with adenoviral vectors, including viral production and infection, be performed under Biosafety Level 2 (BL-2). Adenovirus has been designated as a Level 2 biological agent by the Centers for Disease Control. For more information about the BL-2 guidelines and adenovirus handling, refer to the document, "Biosafety in Microbiological and Biomedical Laboratories", 4th Edition, published by the Centers for Disease Control (CDC). This document may be downloaded from the Web at the following address: <http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm>. A few of the suggested practices include wearing safety glasses, gloves, and lab coats when handling adenoviruses and decontaminating potentially biohazardous wastes before disposal.

Follow federal, state and local regulations for disposal of potentially biohazardous wastes and handle all adenoviruses in compliance with established institutional guidelines. Proper waste contamination should be strictly followed. Since safety requirements for use and handling of adenoviruses may vary at individual institutions, we recommend consulting the health and safety guidelines and/or officers at your institution.

This product is distributed for laboratory use only.

CAUTION: Not for clinical use. The safety and efficacy of this product in clinical uses has not been established.