01
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DATASHEET

pAAV-Rep2Cap2

Cat. No.: RP-B00003

Product Introduction

Description	An AAV packaging plasmid expressing Rep/Cap genes for serotype 2. It is used in combination with a pHelper plasmid and a transgene plasmid (containing the gene of interest flanked by ITRs) to enable high-efficiency AAV packaging. For our catalog, we offer high-grade Tier 2 plasmid quality, optimized for transfection studies. Our plasmids are delivered in ≥80% supercoiled form with ≤0.01 EU/μg endotoxin, ensuring high purity and superior transfection efficiency for reliable AAV vector production.
Turnaround Time	Next-day Shipping

Product Specification

Product Type	AAV Packaging Plasmid Rep2Cap2
Bacteria Resistance	Kanamycin

Quality Control Specifications

Appearance	Clear, colorless, and free from precipitates or foreign particles
Homogeneity (Supercoil)	≥80%
Endotoxin Level	≤0.01 EU/μg
Residual RNA	Not detectable at 200 ng
E. coli Genomic DNA	<15% of all bands via AGE
Restriction Analysis	Conforming to reference pattern
Sequencing	100% Sequence Accuracy
Bio-burden Test	No growth on agar plate after 72 hours

Storage

Storage Conditions	Store at -20°C
Format	5mg/20mg in TE Buffer at 1mg/mL

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Disclaimer

For Research Use Only. Not for use in human therapeutics or diagnostic procedures.

Experimental Protocols

AAV Virus Packaging (293T Adherent Cells)

1. Cell Preparation

Retrieve frozen cells from the liquid nitrogen tank and quickly thaw them in a 37°C water bath.

Centrifuge the cells and resuspend them in fresh culture medium.

Culture the cells at 37°C with 5% CO_2, and passage them every 2-3 days.

Once the cells are growing normally, transfer them to a 10 cm culture dish for adherent culture and set aside for later use.

2. Plasmid Transfection

When the cell density reaches approximately 80-90% confluency, proceed with transfection.

Prepare the three-plasmid transfection system according to a specific ratio（Transfer Vector：pHelper：pRep/Cap=1：1：1）, and transfect the cells using a 1000 μL system per dish.

3. Cell Culture

Generally, a transfection efficiency of over 80% is required. Low transfection efficiency may result in reduced virus yield.

4. Virus Harvest and Debris Removal

After 72 hours of culture, collect the virus supernatant. Centrifuge the virus supernatant at low temperature to remove cell debris. Add an appropriate amount of nuclease and incubate at 37°C to remove free nucleic acids. Then, add PEG8000/NaCl solution and incubate overnight for precipitation.

Target Cell Transduction

It is recommended to use a control adenovirus expressing green fluorescent protein to determine the optimal multiplicity of infection (MOI) for the target cells. MOI refers to the number of viral particles infecting each cell. In other words, an MOI of 1 means that the amount of virus used for each cell can form one plaque-forming unit (PFU).

Protocol of Mammalian Cell Transduction

1. One day before transduction (Day 0)

Seed a certain number of target cells into a new culture dish (the confluence of target cells should be 30%-50% at the time of transduction). Incubate in a 37℃ incubator with 5% CO2 for 18-20 hours. For example, when the target cells are 293T, it is recommended to seed 3×105 cells into one well of a 6-well plate.

2. **On the day of transduction (Day 1)**

1) Thaw the frozen virus stock on ice. Occasionally, the thawed virus may appear slightly turbid, which is normal and does not affect its use. To accurately pipette the virus, gently mix the thawed virus particles before

taking the appropriate amount into the appropriate amount of medium, and then gently mix (avoid vortexing). For better transduction efficiency, use as little medium as possible, just enough to cover the surface of the culture dish. Generally, the amount is about 100 μL/cm². For example, when transducing in a 6-well plate, it is recommended to use 1 mL of medium for one well (the surface area of one well in a 6-well plate is about 10 cm²).

Note: For easily infected cells, the initial MOI for transducing cells can be between 1-100. For more difficult-to-infect cells, a higher MOI may be required.

2) Aspirate the old medium and add the virus-containing medium to the cells.

3) Gently shake the culture plate to ensure that the virus solution covers all the cells. Incubate in a 37℃ incubator with 5% CO2 overnight.

Note: If you are concerned that prolonged exposure of cells to the virus solution may affect cell viability, you can shorten the transduction time to 6-8 hours.

3. Day 2

Change the medium. Aspirate the virus-containing medium and add fresh complete medium. Incubate in a 37℃ incubator with 5% CO2 overnight.

4. Day 3 and beyond

After transduction, analyze the expression of the target gene at the desired time points. Generally, the target gene is significantly expressed 24-48 hours after transduction.

Note: For rapidly dividing cells (with a doubling time of about 24 hours), transgenic expression can be detected within 24 hours after transduction, reaching a maximum at 48-96 hours after transduction (Days 2-4). Five days after transduction, expression levels usually begin to decline. For cell lines with a longer division cycle or no division behavior, high levels of transgenic expression often persist for longer periods. If you are using recombinant adenovirus to transduce mammalian cells for the first time, it is recommended to conduct a time-course experiment to determine the optimal expression time for the target gene.