
Product Manual

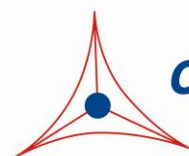
RAPAd[®] CMV Adenoviral Expression System

Catalog Number

VPK-252

1 kit

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Recombinant adenoviruses have tremendous potential in both research and therapeutic applications. There are numerous advantages they provide when introducing genetic material into host cells. The permissive host cell range is very wide. The virus has been used to infect many mammalian cell types (both replicative and non-replicative) for high expression of the recombinant protein. Recombinant adenoviruses are especially useful for gene transfer and protein expression in cell lines that have low transfection efficiency with liposome. After entering cells, the virus remains epichromosomal (i.e. does not integrate into the host chromosome so does not activate or inactivate host genes). Recently, recombinant adenoviruses have been used to deliver RNAi into cells.

Two methods have traditionally been used to generate recombinant adenoviruses. The first involves homologous recombination of a shuttle vector containing gene of interest and an adenoviral backbone plasmid vector (restricted in E1/E3) in an adenovirus packaging cell line. The isolation of recombinant adenovirus by this method involves performing multiple plaque isolations to avoid wild-type virus and is extremely laborious and time consuming. The second method, pAdEasy system, employs the homologous recombination machinery in *E. coli*, a recombinant adenovirus is produced by a double-recombination event between cotransformed adenoviral backbone plasmid vector and a shuttle vector carrying the gene of interest. For the pAdEasy method, the system is high fidelity, but inefficient and requires the screening of many bacterial colonies. This results in a significant time commitment even before transfection of recombinant DNA into E1-expressing cells such as HEK293 cells.

Cell Biolabs' RAPAd® Adenoviral Expression System provides a much faster and safer method to generate RCA-free recombinant adenovirus at high titer (see Table 1). The RAPAd® system uses a novel Ad backbone devoid of the left-hand ITR, the packaging signal and E1 sequences. There is no need to perform the bacterial *in vitro* homologous recombination (pAdEasy method), and also the multiple plaque isolations (standard homologous recombination method in packaging cell line). The RAPAd® system allows for generation of a recombinant virus within 2 weeks and the virus produced contained virtually no contaminating E1a sequences or replication-competent virus (RCA).

Cell Biolabs' RAPAd® Adenoviral Expression System is simple to use. The method is straightforward and requires very limited 'hands on' time from shuttle/backbone cotransfection to the isolation of virus particles. It produces equivalent infectious titers as the standard viral genome/shuttle plasmid recombination method.

In Cell Biolabs' RAPAd® CMV Adenoviral Expression System, the shuttle vector contains a CMV ahead of the multiple cloning sites.

Standard Homologous Recombination	pAdEasy Expression System	RAPAd® Expression System
<p data-bbox="155 327 493 428">Cotransfect 293 cells with Shuttle Vector and Ad Backbone Vector</p> <p data-bbox="310 464 326 604">↓</p> <p data-bbox="261 621 380 722">Multiple Plaque Isolations</p> <p data-bbox="310 751 326 892">↓</p> <p data-bbox="233 909 412 980">Virus Amplification</p> <p data-bbox="310 989 326 1129">↓</p> <p data-bbox="250 1167 396 1197">Viral Stock</p>	<p data-bbox="610 327 799 428">Linearize Shuttle Vector using PmeI</p> <p data-bbox="691 464 708 604">↓</p> <p data-bbox="526 621 883 722">Cotransform <i>E.coli</i> BJ5183 cells with linearized Shuttle Vector and pAdEasy Vector</p> <p data-bbox="691 751 708 892">↓</p> <p data-bbox="529 909 880 980">Recombinant selection by restriction enzyme analysis</p> <p data-bbox="691 989 708 1129">↓</p> <p data-bbox="558 1167 850 1239">Linearize recombinant plasmid using PacI</p> <p data-bbox="691 1247 708 1388">↓</p> <p data-bbox="581 1425 828 1455">Transfect 293 cells</p> <p data-bbox="691 1484 708 1625">↓</p> <p data-bbox="630 1642 779 1671">Viral Stock</p>	<p data-bbox="915 327 1281 428">Linearize Shuttle Vector and RAPAd® Ad Backbone Vector using PacI</p> <p data-bbox="1097 464 1114 604">↓</p> <p data-bbox="1024 621 1175 680">Cotransfect 293 cells</p> <p data-bbox="1097 751 1114 892">↓</p> <p data-bbox="1024 909 1175 938">Viral Stock</p>
12-18 weeks	8-9 weeks	2-3 weeks

Table 1. Outline of Recombinant Adenovirus Systems

Related Products

1. AD-100: 293AD Cell Line
2. AD-200: ViraDuctin™ Adenovirus Transduction Reagent
3. VPK-099: ViraBind™ Adenovirus Miniprep Kit
4. VPK-109: QuickTiter™ Adenovirus Titer Immunoassay Kit
5. VPK-111: Rapid RCA Assay Kit

Kit Components (shipped on blue ice)

1. pacAd5 CMVK-NpA Shuttle Vector (Part No. 325201): One 40 µL vial at 0.25 mg/mL.
2. pacAd5 9.2-100 Vector (Part No. 325002): One 40 µL vial at 0.25 mg/mL.
3. pacAd5 CMV-GFP Control Vector (Part No. 325004): One 40 µL vial at 0.25 mg/mL.
4. pacAd5 CMV-ntLacZ Control Vector (Part No. 325202): One 40 µL vial at 0.25 mg/mL.

Materials Not Supplied

1. 293 cells: we recommend 293AD Cell Line (Cat.# AD-100) for high titer production of recombinant adenovirus.
2. 293 Cell Culture Medium
3. Transfection Reagents
4. PacI (New England Biolabs, Cat.# R0547L)

Storage

Upon receipt, store all kit components at -20°C.

Safety Considerations

Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms.

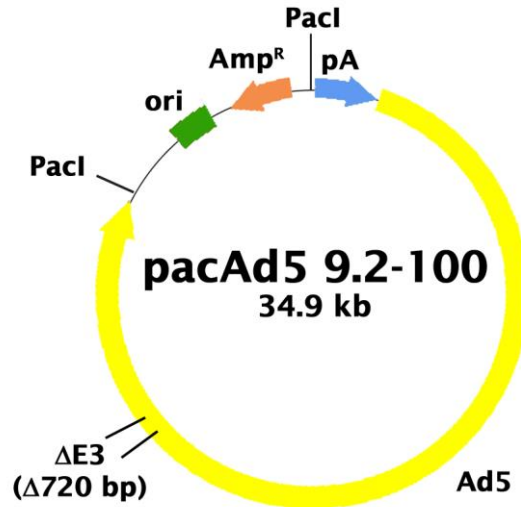


Figure 2. pacAd5 9.2-100 Vector (34947 bp, Ampicillin-resistant). The novel pacAd5 9.2-100 Ad backbone vector is devoid of the left-hand ITR, the packaging signal and E1 sequences.

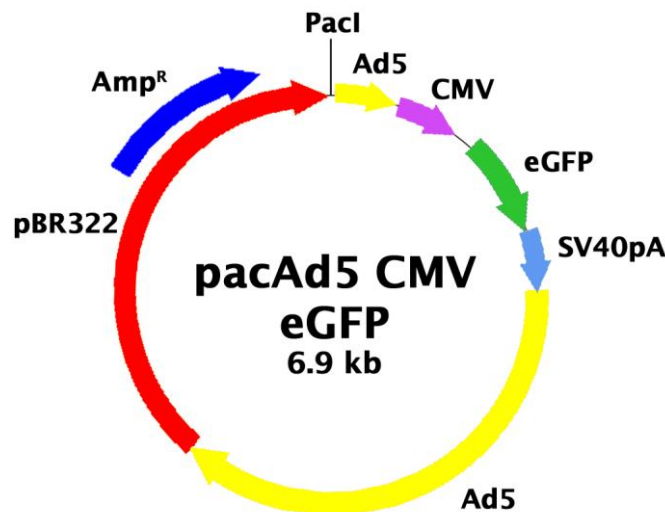


Figure 3. pacAd5 CMV-GFP Control Vector (6935 bp, Ampicillin-resistant).

pacAd5 CMV-GFP Features:

3-10:	PacI
16-368:	1-353 of Ad5
385-912:	CMV Promoter
992-1711:	GFP
1713-2160:	SV40 pA
2161-4615:	3328-5792 of Ad5
5867-6727:	β Lactamase

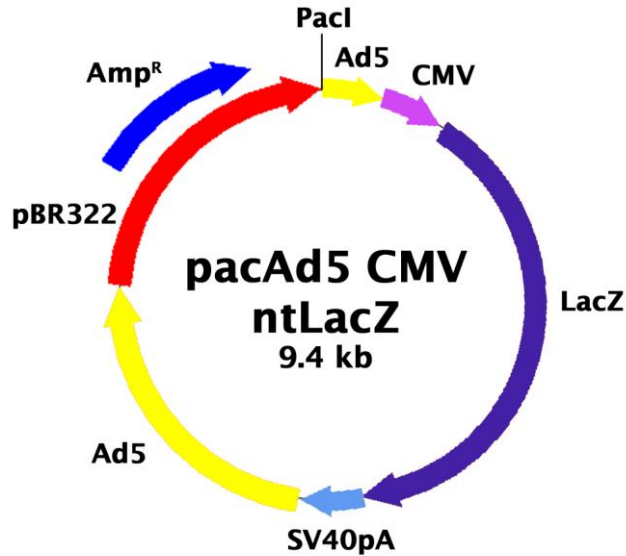


Figure 4. pacAd5 CMV-ntLacZ Control Vector (9278 bp, Ampicillin-resistant).

pacAd5 CMV-ntLacZ Features:

3-10:	PacI
16-368:	1-353 of Ad5
385-912:	CMV Promoter
1105-4148:	ntLacZ
4193-4640:	SV40 pA
4641-7095:	3328-5792 of Ad5
8347-9210:	β Lactamase

Preparation of Recombinant Adenovirus

I. Vector Linearization with PacI

1. Digest a sufficient amount of the pacAd5 CMV-K-NpA shuttle vector containing gene of interest and the pacAd5 9.2-100 Ad backbone vector with PacI.
2. Run 0.5 μ g of each digested DNA and undigested DNA on a 0.8% agarose gel to confirm the completion of PacI digestion (For pacAd5 9.2-100, one band of ~33 kb and a second band of 2.0 kb).
3. Remove buffer and enzyme from the remainder of the restriction reactions by phenol extraction/ethanol precipitation or using a similar DNA purification kit.
4. Resuspend the DNA in sterile dH₂O. Store the digested DNA at -20°C.

II. Transfection

1. Seed 2 x 10⁶ cells in a 60 mm culture dish without antibiotics one day before transfection.
2. After 16 to 24 hours, start transfection when the culture becomes 70-80% confluence.
Note: We suggest transfecting cells with FuGENE® Transfection Reagent (Roche Applied

Science) or Lipofectamine™ Plus (Invitrogen). For example, 4 µg of pacAd5 CMVK-NpA shuttle vector and 1 µg of pacAd5 9.2-100 Ad backbone vector are mixed with 9 µL FuGENE® Transfection Reagent according to the manufacturer's recommendation. The mixed DNA-FuGENE® complex is added by dropwise into the culture media.

3. Aspirate the media containing transfection reagent the next day and add 4 mL of complete culture medium.
4. After incubating for 7 days, check for the presence of plaques. If plate is ready for harvest, (>50% of cells lifted), then collect the Crude Viral Lysate.
5. If not, feed the cells with 1 mL of complete culture medium, continue to incubate at 37°C with CO₂.
6. On day 10, check for the presence of plaques. If plate is ready for harvest, (>50% of cells lifted), then collect the Crude Viral Lysate.
7. If not, feed the cells with 1 mL of complete culture medium, continue to incubate at 37°C with CO₂. Keep checking plate for the presence of plaques. Do not keep plate more than 15 days.

III. Harvesting the Crude Viral Lysate

1. Harvest adenovirus-containing cells by squirting cells off the plate with a 5 or 10 mL sterile serological pipette. Transfer cells and media to a sterile 15 ml tube. Scraping the cells into the medium with a cell lifter if necessary.
2. Release viruses from cells by three freeze/thaw cycles (10 minutes each in 37°C water bath and dry ice-methanol bath).
3. Centrifuge the cell lysate in a table-top centrifuge at 3000 rpm for 15 minutes at room temperature to pellet the cell debris.
4. Aliquot and store the Crude Viral Lysate (Initial Viral Stock) at -80°C.

IV. Amplification

Note: The following procedure is suggested for T75 flasks and may be optimized to suit individual needs.

1. Seed 3-5 x 10⁶ cells in a T75 flask one day before infection.
2. Add 50% of the above Crude Viral Lysate to the culture. We recommend using a multiplicity of ≥0.5 PFU (plaque forming units) or enough viruses that cells demonstrate cytopathic effects (CPEs) within 48 hrs.
3. During 24 - 48 hr infection, examine the monolayer twice per day under the microscope for CPE. When CPE is nearly complete (i.e. most cells rounded but not yet detached from the flask), harvest cells by pipetting media up and down to wash the infected cells from the flask into the media.
4. Pool infected cells and medium. Pellet cells by centrifugation at 1000 g for 5 minutes. Remove supernatant, resuspend cell pellet in medium or in 10 mM Tris, pH 8.0, 100 mM NaCl. (0.25-0.5 mL per T75 flask).
5. Release the adenoviruses from the cell suspension with three freeze/thaw cycles. Centrifuge at 3000 g for 10 minutes to pellet the cell debris. Discard the pellet and save supernatant as viral stock.
6. The viral supernatant can be stored at -80°C or immediately purified or titered.

Example of Results

The following figures demonstrate typical results of generating recombinant adenovirus. One should use the data below for reference only. This data should not be used to interpret actual results.

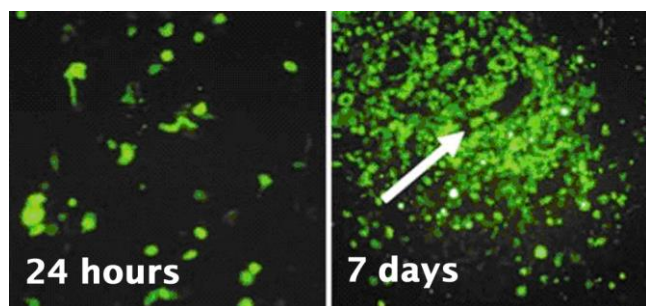


Figure 5. Generation of recombinant adenovirus using the RAPAd® Adenoviral Expression System. 293 cells were transfected with PacI linearized pacAd5 CMV-GFP vector and pacAd5 9.2-100 vector. Plates were examined for the presence of viral foci under inverted fluorescence microscope.

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Recent Product Citations

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Contact Information

Cell Biolabs, Inc.
5628 Copley Drive
San Diego, CA 92111
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

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