Product Manual

PureVirus™ Adenovirus Purification Kit

Catalog Number

VPK-5112 **10 preps**

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 in using an adenovirus to introduce 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.

HEK 293 cells or their variants are used as host cells for viral amplification. Recombinant adenoviruses can be grown at high titer (10^{10} VP (viral particles)/mL, which can be concentrated up to 10^{13} VP/mL). The concentrated viral supernatant is subjected to CsCl ultracentrifugation to separate the viruses from the cellular proteins and media components. Following ultracentrifugation, CsCl is then removed by dialysis. The CsCl procedure is both tedious and time consuming (16-24 hrs).

Cell Biolabs' PureVirus™ Adenovirus Purification Kit does not require ultracentrifugation. Instead, host cell protein and nucleic acid contaminants are captured and removed through the purification resin. Each prep is designed to purify viruses harvested from up to four T75 flasks or 10-cm dishes. The purification protocol takes less than 2 hours.

The PureVirus™ Adenovirus Purification Kit provides an efficient system for the quick removal of >98% of host cell proteins, nucleic acids and other contaminants, with a high recovery of virus (>60-70%).

Related Products

1. AD-100: 293AD Cell Line
2. AD-200: ViraDuctin™ Adenovirus Transduction Reagent, 10 transductions
3. AD-201: ViraDuctin™ Adenovirus Transduction Reagent, 50 transductions
4. VPK-109: QuickTiter™ Adenovirus Titer Immunoassay Kit
5. VPK-110: QuickTiter™ Adenovirus Titer ELISA Kit
6. VPK-111: Rapid RCA Assay Kit
7. VPK-252: RAPAd® CMV Adenoviral Expression System

Kit Components

1. Virus Purification Resin (Part No. 51121B): One 8 mL bottle containing virus purification resin (50% slurry) in 20 mM Tris, pH 8.0, 25 mM NaCl.
2. 100X Virus Pretreatment Solution (Part No. 51122B): One 200 µL tube.
3. Virus Purification Buffer (Part No. 51123A): One 10 mL bottle containing 20 mM Tris, pH 8.0, 25 mM NaCl.
4. Microspin Columns (Part No. 51124A): 10 columns.

Materials Not Supplied

1. Recombinant adenovirus of interest
2. HEK 293 cells and cell culture growth medium
3. Cell culture centrifuge
4. Glycerol
5. 100 kD MWCO Concentrator
6. Microcentrifuge

Storage

Store the Virus Purification Buffer and Microspin Columns at room temperature and all other kit components at 4ºC.

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.

Preparation of Reagents

- Virus Lysis Buffer: Prepare fresh Virus Lysis Buffer by diluting the provided 100X Virus Pretreatment Solution 1:100 in Virus Purification Buffer (e.g. Add 10 µL of 100X Virus Pretreatment Solution to 1.0 mL of Virus Purification Buffer).

  Note: Prepare only the amount of Virus Lysis Buffer to be used immediately.

Harvesting Infected Cell Lysate

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

1. Use HEK 293 cells that have been passaged regularly 2-3 times prior to the infection. Culture these cells until the monolayer is 90-100% confluent.
2. Replace the cell culture media with new growth media, 15 mL per flask. Next, the adenovirus is added to the culture. Either crude or purified viral stock can be used. A multiplicity of 0.5 to 2 PFU (plaque forming units) per cell is desired.
3. After 24 hours, some cells should be floating. Add 10 mL growth media to the culture flask and allow the viruses to expand for another 24 hours. When all the cells are floating, gently shake the culture flask several times and harvest all media, including cells, in a sterile tube.
4. Centrifuge at 1000 rpm for 5 minutes to pellet the cells. Resuspend the cell pellet in 0.5 mL of freshly prepared Virus Lysis Buffer. Incubate 30 min at 37ºC, and then fully release the adenoviruses from the cells with three freeze/thaw cycles.
5. Centrifuge at 10,000 rpm for 10 minutes. Transfer supernatant into another microcentrifuge tube and discard the cell debris.

6. The viral lysate supernatant can be stored at -80ºC or immediately purified (see Purification Protocol below).

Purification Protocol

1. Mix the Virus Purification Resin well by vortex. Add 0.4 mL of Virus Purification Resin (50% slurry) to each of two microcentrifuge tubes and centrifuge at 10,000 rpm for 1 minute. Carefully remove and discard the supernatant from both tubes.
2. Add 0.5 mL of viral lysate supernatant to one microcentrifuge tube containing just the Virus Purification Resin (from Step 1). Mix well and incubate 30 min on an orbital shaker at room temperature.
3. Centrifuge at 10,000 rpm for 1 minute. Transfer the supernatant to the second microcentrifuge tube containing just the Virus Purification Resin (from Step 1). Mix well and incubate 30 min on an orbital shaker at room temperature.
4. Centrifuge at 10,000 rpm for 1 minute. Transfer the supernatant containing purified virus to an empty Microspin Column and centrifuge at 10,000 rpm for 1 min to clear any remaining Virus Purification Resin.
5. (optional) Concentrate virus by 100 kD MWCO concentrator.
6. Add glycerol to a final concentration of 10% to the purified virus or dialyze the viral solution into a desired buffer. Aliquot and store the final purified virus solution at -80ºC.

Example of Results

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

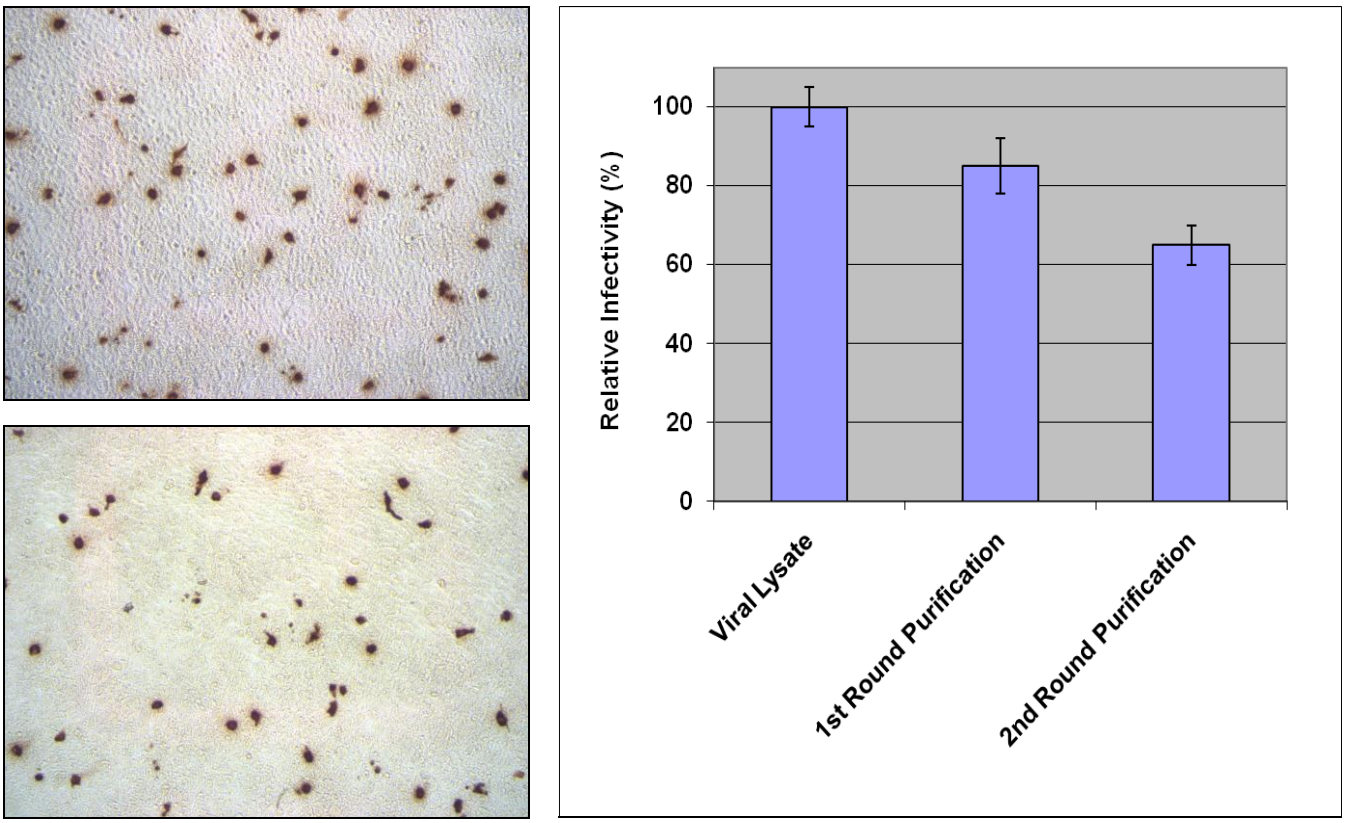

Figure 1: **Purification of recombinant Ad-β Gal.** Recombinant Ad-β Gal viruses were purified according to the Purification Protocol. The adenoviral infectivity of each fraction obtained during purification was determined through Hexon staining using QuickTiter™ Adenovirus Titer Immunoassay Kit (Cat. #VPK-109). Top Left: Viral Lysate Supernatant; Bottom Left: Purified Adenovirus Sample. Removal of host cell protein and nucleic acid contaminants were monitored through BCA protein assay and CyQuant DNA/RNA assay. More than 98% of both host cell proteins and nucleic acids were removed in the final purified adenovirus sample (data not shown).

References

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Recent Product Citations

1. Das, D. et al. (2021). Proteasome dysfunction under compromised redox metabolism dictates liver injury in NASH through ASK1/PPARγ binodal complementary modules. *Redox Biol*. doi: 10.1016/j.redox.2021.102043.
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3. Cho, J.H. et al. (2020). CD9 induces cellular senescence and aggravates atherosclerotic plaque formation. *Cell Death Differ*. doi: 10.1038/s41418-020-0537-9.

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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