Product Manual

ViraBind™ Adenovirus Miniprep Kit

Catalog Number

VPK-099 **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). ViraBind™ Adenoviral Miniprep Kit does not involve ultracentrifugation, instead viruses are captured on spin column based on the unique properties of adenoviral capsid proteins. The entire procedure takes about 30 minutes. Each column is designed to purify viruses harvested from one T75 flask or 10-cm plate, and has a capacity of up to 1.0 x 10^{11} VPs.

ViraBind™ Adenovirus Miniprep Kit provides an efficient system for quick adenoviral purification with high recovery (>95%). The system may be adapted to purification of other viral types, such as retrovirus and lentivirus.

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. ViraBind™ Columns and Collection tubes (Part No. 40991): Pack of 10 mini spin columns and 20 collection tubes.
2. Loading Buffer (Part No. 40992): One 10 mL bottle.
3. Wash Buffer (Part No. 40993): One 20 mL bottle.

4. Elution Buffer (Part No. 40994): One 10 mL bottle of 25 mM Tris, pH 7.5, 2.5 mM Mg_2Cl, 1 M NaCl.

Materials Not Supplied

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

Storage

Store all kit components at room temperature.

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.

Harvesting Infected Cell Lysate

The following procedure is suggested for a T75 flask 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 hrs, some cells should be floating. Add 10 mL growth media to the culture flask and allow the viruses to expand for another 24 hrs. 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 0.8 mL of culture medium and release the adenoviruses from the cells with three freeze/thaw cycles. Transfer supernatant into another microcentrifuge tube and discard the cell debris.
5. Centrifuge at 10,000 rpm for 10 minutes to further pellet any remaining cell debris. Discard the pellet and save supernatant. If a large amount of cell debris is still visible, centrifuge the supernatant again.

Note: This step is useful to prevent clogging of the spin columns during the virus purification steps.

6. The viral supernatant can be stored at -80ºC or immediately purified (see purification instructions below).

Purification Protocol

1. Add 0.4 mL of Loading Buffer to the ViraBind™ spin column and spin for 5 minutes at 2000 g. Discard the flow-through.
2. Apply 0.4 mL of viral supernatant to the ViraBind™ spin column and spin for 5 minutes at 2000 g. Discard the flow-through. Load the remaining viral supernatant and discard the flow-through.
3. Wash the ViraBind™ spin column by adding 0.4 mL of Wash Buffer and centrifuging for 5 minutes at 2000 g. Discard the flow-through. Repeat once the wash and spin step.
4. Place the ViraBind™ spin column in a clean collection tube. To elute virus, add 0.4 mL of Elution Buffer (25 mM Tris, pH 7.5, 2.5 mM Mg_2Cl, 1 M NaCl) and centrifuge for 5 minutes at 2000 g.
5. 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.

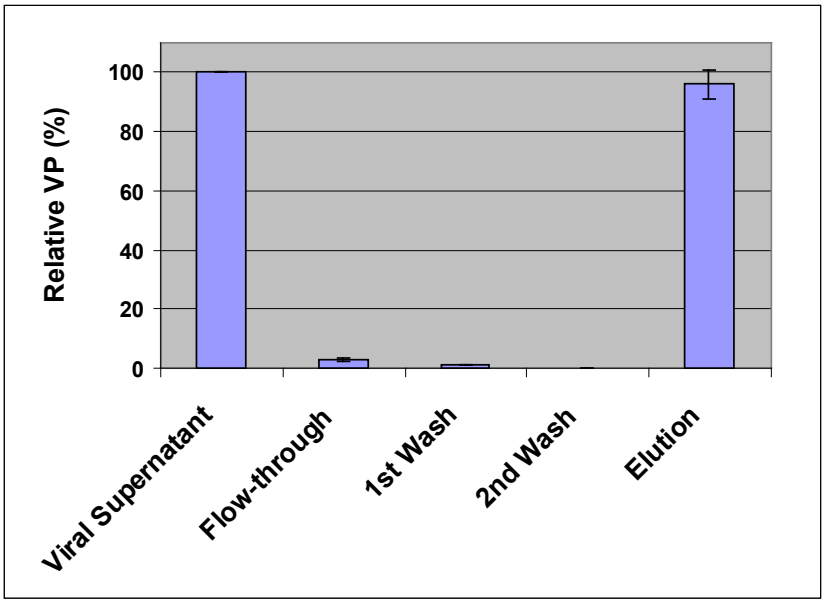

Figure 1: **Purification of recombinant Ad-β Gal.** Recombinant Ad-β Gal viruses were purified according to the Purification Instructions. Each fraction, obtained during purification, and its dilution were used to infect A549 cells in a 12-well plate. After 48 hrs, X-gal staining was performed and β Gal positive cells (blue) were scored.

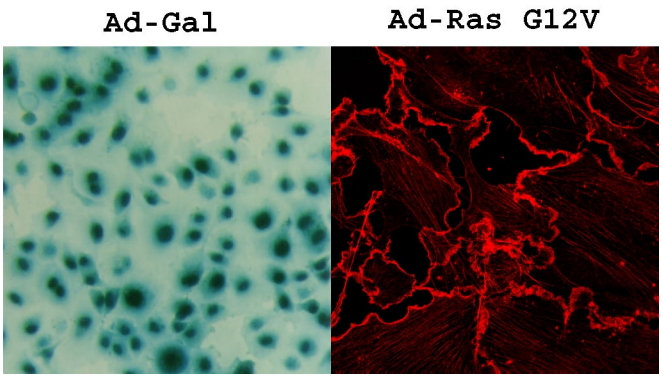

Figure 2: Membrane Ruffling Induced by Activated H-Ras. COS-7 cells were infected with purified Ad-β Gal or Ras G12V at 50 MOI (multiplicity of infection). X-gal staining was performed after 48 hr infection period. Membrane ruffling is visualized by staining the actin cytoskeleton with Rhodamine-coupled Phalloidin.

References

1. Stewart, P. L., Fuller, S. D., and Burnett, R. M. (1993) *EMBO J.* 12, 2589-2599.
2. Robbins, P. D., Tahara, H., and Ghivizzani, S. C. (1998) *Trends Biotechnol.* 16, 35-40.
3. Huang, S., Stupack, D., Mathias, P., Wang, Y., and Nemerow, G. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 8156-8161.
4. Bergelson, J. M., J. A. Cunningham, G. Droguett, E. A. Kurt-Jones, A. Krithivas, J. S. Hong, M. S. Horwitz, R. L. Crowell, and R. W. Finberg. (1997) *Science* 275:1320-1323.
5. Von Seggern, D. J., C. Y. Chiu, S. K. Fleck, P. L. Stewart, and G. R. Nemerow. (1999) *J. Virol.* 73:1601-1608.
6. Kleinerman, D. I., W. W. Zhang, S. H. Lin, N. T. Van, A. C. von Eschenbach, and J. T. Hsieh. (1995) *Cancer Res*. 55:2831-2836.

Recent Product Citations

1. Pang, L. et al. (2021). Attenuation and transport of human enteric viruses and bacteriophage MS2 in alluvial sand and gravel aquifer media-laboratory studies. *Water Res*. **196**:117051. doi: 10.1016/j.watres.2021.117051
2. Watanabe, K. et al. (2021). ILDR2 stabilization is regulated by its interaction with GRP78. *Sci Rep*. **11**(1):8414. doi: 10.1038/s41598-021-87884-7.
3. Teves, M.E. et al. (2020). Human DENND1A.V2 Drives Cyp17a1 Expression and Androgen Production in Mouse Ovaries and Adrenals. *Int J Mol Sci*. **21**(7). pii: E2545. doi: 10.3390/ijms21072545.
4. Watanabe, K. et al. (2019). Kbtbd11 contributes to adipocyte homeostasis through the activation of upstream stimulatory factor 1. *Heliyon*. doi: 10.1016/j.heliyon.2019.e02777.
5. Lochbaum, R. et al. (2019). Retinoic acid signalling adjusts tight junction permeability in response to air-liquid interface conditions. *Cell Signal*. doi: 10.1016/j.cellsig.2019.109421.
6. Watanabe, K. et al. (2019). A novel upstream transcription factor 1 target gene N4bp2l1 that regulates adipogenesis. *Biochemistry and Biophysics Reports*. doi: 10.1016/j.bbrep.2019.100676.

7. Putranto, E.W. et al. (2017). Expression of tumor suppressor REIC/Dkk-3 by a newly improved adenovirus vector with insertion of a hTERT promoter at the 3'-side of the transgene. *Oncology Letters* **14**:1041-1048.
8. Xie, Y. et al. (2016). A recombinant adenovirus expressing P12A and 3C protein of the type O foot-and-mouth disease virus stimulates systemic and mucosal immune responses in mice. *BioMed Res Int*. doi:10.1155/2016/7849203.
9. Morris, S. J. et al. (2015). Laboratory-scale production of replication-deficient adenovirus vectored vaccines. *Vaccine Technol Vet Viral Disease.* doi:10.1007/978-1-4939-3008-1_8.
10. Müller, J. et al. (2015). TRIM21, a negative modulator of LFG in breast carcinoma MDA-MB-231 cells in vitro. *Int J Oncol.* **47**:1634-1646.
11. Zhu, L. et al. (2015). Inhibition of porcine reproductive and respiratory syndrome virus replication with exosome-transferred artificial microRNA targeting the 3′ untranslated region. *J Virol Methods.* **223**:61-68.
12. Boehme, P. et al. (2015). Standard free droplet digital polymerase chain reaction as a new tool for the quality control of high-capacity adenoviral vectors in small-scale preparations. *Hum Gene Ther Methods.* **26**:25-34.
13. Zhu, L. et al. (2014). Inhibition of porcine reproductive and respiratory syndrome virus infection by recombinant adenovirus-and/or exosome-delivered the artificial microRNAs targeting sialoadhesin and CD163 receptors. *Virol J.* **11**:225.
14. Chen, Y. et al. (2014). Additive inhibition of porcine reproductive and respiratory syndrome virus infection with the soluble sialoadhesin and CD163 receptors. *Virus Res.* **179**:85-92.
15. McAllister, J.M. et al. (2014). Overexpression of a DENND1A isoform produces a polycystic ovary syndrome theca pehontype. *PNAS* **111**:E1519-E1527.
16. Wilkins, H. et al. (2013). Mitochondrial glutathione transport is a key determinant of neuronal susceptibility to oxidative and nitrosative stress. *J. Biol. Chem*. **288**:5091-5101.
17. Wang, Y.S. et al. (2012). MicroRNA-195 regulates vascular smooth muscle cell phenotype and prevents neointimal formation. *Cardiovasc Res*. **95**:517-526.
18. Kirui, J.K. et al. (2010). Gßgamma signaling promotes breast cancer cell migration and invasion. *J. Pharmacol. Exp. Ther*. **333**:393-403.

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