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Fast, Effective and Safe Adenovirus Purification with Vivapure® Adenopack Kits

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Abstract

The incorporation of Sartobind® membrane adsorbers into Vivapure® Adenopack units facilitates and accelerates the purification step for adenovirus preparation, which can take days using conventional methods. Ready-to-use kits are available for processing initial cell culture volumes from 20 to 500 mL, permitting the preparation of up to 3×10^{13} purified viral particles in only 2 hours. Furthermore, the method can be conveniently transferred to Sartobind® capsules and cassettes, for adenovirus purification at process scale.

Introduction

Recombinant viral vectors are used to infect cells for expression of specific proteins in signal transduction experiments, gene-function analysis or gene-therapy research and development. Adenovirus is a nonenveloped dsDNA virus with a diameter of 80–90 nm. Owing to its ability to infect most mammalian cell types (replicating and non-replicating) and to replicate at high titers, it is one of the most widespread systems for viral gene transfer. Various requirements need to be met when purifying adenovirus from cell culture for the above-mentioned applications. In the early phases of a research project, many different adenoviral constructs need to be screened. For this purpose, only small virus amounts are required. Gene-therapy experiments, however, require large quantities of virus. Additionally, the possibility of scaling up the method is essential when entering Phase I experiments (Volpers and Kochanek, 2004).

Adenovirus is cultivated in HEK 293 cells and purified from the cell lysate as soon as cells start to exhibit cytopathic effects. The most common purification method uses a cesium chloride (CsCl) density gradient, in which the adenovirus is purified according to its specific density. Density gradients, followed by the obligatory dialysis step to remove cytotoxic CsCl, are very time-consuming and difficult to pursue with both small and large-volume cell cultures. Because adenovirus is negatively charged under physiological conditions, it can effectively be purified using ion exchange chromatography (Delmdahl, 2006).

Membrane chromatography offers a simple, scalable, and fast alternative to the lengthy CsCl gradient purification method, as well as purification with traditional chromatographic resins or beads. Membrane adsorbers are highly porous, with pore sizes exceeding 3,000 nm, and thus offer rapid, unhindered access of larger targets such as adenoviral particles to their charged surface (Demmer and Nussbaumer, 1999). Figure 1 shows an electron micrograph of a membrane adsorber in comparison to common chromatography beads. Distinct, large pores are clearly visible on the membrane adsorber, in contrast to the dense bead-surface structure.

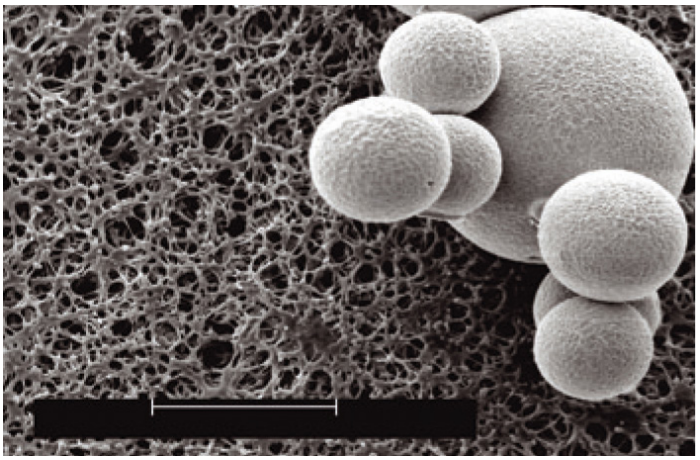

Figure 1: *Electron Micrograph Comparing the Open Porous Structure of a Sartobind® Membrane Adsorber (Background) to the Much Smaller Pores of Chromatography Beads (Foreground). Scale Bar, 50 µm.*

Vivapure® Adenopack kits include a ready-to-use membrane adsorber for convenient adenovirus purification at the laboratory scale. They contain all the filters, buffers, enzymes and other accessories needed, optimized for purification of the most frequently used type 5 adenovirus. A syringe filter format incorporates the membrane adsorber for convenient equipment-free or peristaltic pump-driven purifications.

For a side-by-side comparison, we used Vivapure® Adenopack 100 or a CsCl gradient for the purification of Ad-GFP (adenovirus expressing green fluorescent protein) from 200 mL cell cultures.

Materials and Methods

In both experiments, low-passage HEK 293 cells were infected with the adenovirus stock at a multiplicity of infection (MOI) of 10–20 in several 15 cm plates, each containing 20 mL of Dulbecco's Modified Eagle Medium (DMEM). Cells were collected when they started showing cytopathic effects. This step is not as critical when using the Adenopack kits, since the cell lysate and supernatant are both used for isolating the virus. In contrast, when using CsCl gradients, the cells must be collected before they release the virus into the supernatant, as only the cell pellet is used for purification.

For purification with Adenopack, the harvested cell pellet was resuspended in 10 mL of DMEM and lysed by three freeze-thaw cycles, while the ~180 mL supernatant (which also contains adenovirus particles) was stored on ice.

Following lysis, the sample was centrifuged to remove cell debris and pooled with the supernatant from the previous step. DNA digestion was performed on the entire sample (195 mL) using the included Benzonase® nuclease, followed by a final filtration to prevent blocking of the Adenopack units. A second pellet obtained from 200 mL cell culture was purified by CsCl density gradient according to published protocols (16 h at 100,000 g, after which the viral band was recovered by syringe, followed by a further CsCl density gradient for 16 h at 30,000 g).

Adenopack units were equilibrated with loading buffer before applying the previously prepared sample. At this stage, adenovirus particles were bound by the membrane adsorber ion exchange ligands. Non-specifically bound cell proteins were removed in a wash step before elution of the purified adenovirus. Virus particles were quantified by absorbance measurement at 260 nm, taking one absorbance unit at 260 nm to be equivalent to 10^{12} particles. In addition, the number of infectious particles was quantified using a commercially available kit, virus purity was determined by western blot, and the viral preparation was further assessed by transfection assay for GFP expression in HeLa cells.

Results

Purification by the Adenopack kit yielded 3.5×10^{12} viral particles. In comparison, only 1.5×10^{12} viral particles were purified when using a CsCl density gradient. Infectivity was determined to be equivalent for both methods, with 5×10^{10} infectious particles detected in each purified sample.

Analysis by western blot (Figure 2a) illustrates the high purity of adenovirus when prepared by each method. Following purification by Adenopack and transfection into HeLa cells, microscopy confirmed that the Ad-GFP preparation was highly infectious, as evidenced by the high number of cells exhibiting green fluorescence (Figure 2b).

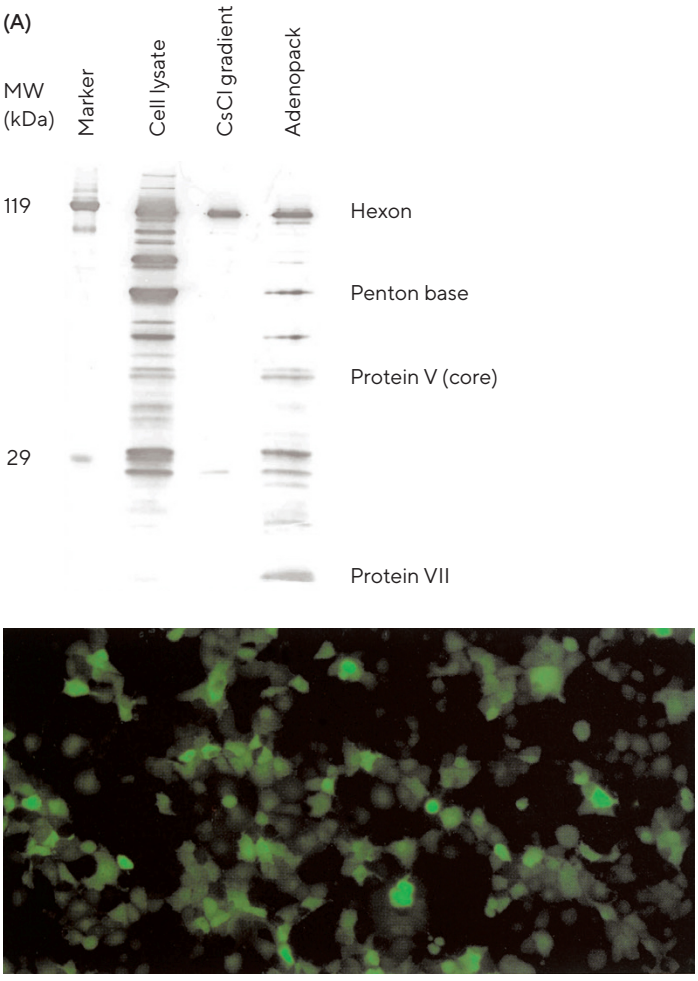

Figure 2: *Analysis of Samples Purified by Vivapure® Adenopack and CsCl density Gradient.*
Note: A. shows a western blot comparing purification effectiveness between the two methods. Detection was via polyclonal rabbit antibody against Ad5 hexon proteins and a secondary goat anti-rabbit-alkaline phosphatase conjugate. B. demonstrates infection of HeLa (green cells) by Ad-GFP purified using Adenopack (image courtesy of G. L. Lux, University Hospital and University of Applied Sciences, Mannheim, Germany).

Conclusions

We have demonstrated that Vivapure® Adenopack kits can provide similar purification results to those obtained via CsCl gradient purification, with higher yields and in only 2 hours - a fraction of the time needed for a purification by CsCl gradient. A transfection assay with purified Ad-GFP in HeLa cells shows that adenovirus purified with the Adenopack is highly infective, and microscopic analysis to determine cell viability (data not shown) clearly indicated high purity and low levels of endotoxin in the pure viral concentrates.

Membrane adsorbers are ideal for virus purification, since the much larger pore structure when compared to chromatography resins allow unrestricted access to the binding ligands and convective rather than diffusive transport speeds up the purification process. Moreover, the method can be easily adapted for adenovirus purification in process scale, using Sartobind® products, which have been developed around the same membrane adsorber platform.

References

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Note

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