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Improved Method for Adenovirus and Lentivirus Purification using the Fast-Trap™ Virus Purification and Concentration Kit

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Abstract

Highly purified virus is necessary for many viral vector applications such as vaccine production and genetic modification. Crude virus preparations often contain cellular debris and proteins from culture media that are toxic to target cells and can cause immunogenic reactions *in vivo*. Conventional virus purification methods based on sucrose or cesium gradient ultracentrifugation are time-consuming, difficult, and require access to special instrumentation. These methods also often result in low virus recovery.

Here we report a fast and easy method for adenovirus and lentivirus purification using the EMD Millipore Fast-Trap™ Virus Purification and Concentration Kit. Both adenovirus (Cat. No. FTAV00003) and lentivirus (Cat. No. FTLV00003) kits contain the necessary reagents and filter devices to accommodate the entire virus purification workflow (Figure 1). The purification is membrane-based and utilizes a closed vacuum-driven device. High recoveries of purified viable viral particles are typically achieved in under two hours.

Introduction

Viruses are an increasingly valuable tool in bioresearch. A variety of virus-derived vectors have been developed for improved gene transfer in mammalian cells. Recombinant viral vectors have widespread use in the field of gene therapy[1] and for vaccination purposes[2]. For both adenovirus and lentivirus, purifying the virus after it is propagated in the host cell is a critical step.

Adenoviral and lentiviral vectors have traditionally been purified by density gradient centrifugation[3,4]. However, density gradient techniques are lengthy, tedious, and often result in low virus recovery. In addition, this purification method requires the use of ultracentrifuges which are expensive and not common equipment in an average laboratory.

Recently, column or membrane chromatography methods[5,6] were developed for virus purification. The amount of time required to complete these methods is much less than by traditional methods, typically under two hours. Most lab-scale chromatographic virus purification devices are syringe filter- or column-based. However, syringe filter-based methods require assembly and disassembly which can lead to messy and potentially hazardous handling conditions.

Here we report a significantly improved solution for virus purification. A new membrane was developed to selectively bind and elute adenoviruses and lentiviruses using specially formulated buffers For added safety and ease of handling, this membrane is housed in a newly engineered, vacuum-based device: the Fast-Trap™ Virus Purification filter unit. Both Adenovirus and Lentivirus Fast-Trap™ Purification kits perform as good as or better than similar products from other manufacturers in terms of processing time, recovery, purity, and capacity. The entire protocol, including clarification of crude virus, purification of the virus, buffer exchange, and concentration, can be accomplished in under two hours and results in a concentrated, high titer, pure virus in the buffer of choice.

EMD Millipore is a division of Merck KGaA, Darmstadt, Germany.

Lentivirus / Adenovirus Preparation and Harvest ► Benzonase Nuclease Enzyme Treatment (Optional)

▼

Fast-Trap™ Lentivirus and Adenovirus Purification & Concentration Kit

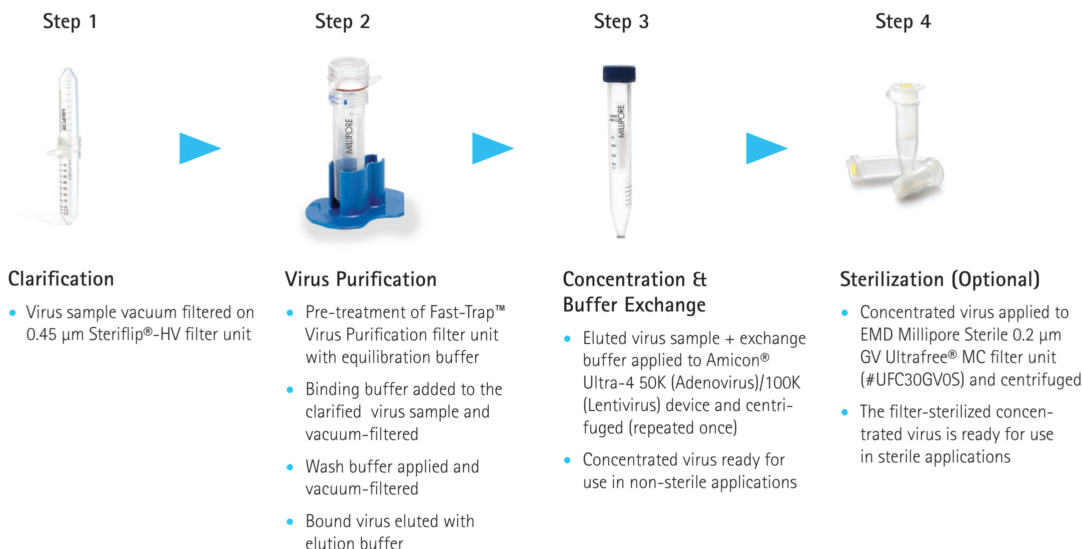


Figure 1.
Virus purification workflow using the Fast-Trap™ Adenovirus (Cat. No. FTAV00003) and Lentivirus (Cat. No. FTLV00003) Purification and Concentration Kits.

Results & Discussion

Adenovirus Purification

Ad5.CMV5-GFP (Q-BIOgene, Cat. No. ADV0032), a serotype 5 adenovirus expressing GFP under the control of a modified CMV promoter, was purified using the components of EMD Millipore's Fast-Trap™ Adenovirus Purification and Concentration Kit.

Ad5.CMV5-GFP viral stock was amplified in HEK293 cells. Two to four days after infection, when cells detach and round up, the cells were harvested and pelleted. Cell pellets were resuspended in fresh media and subjected to three freeze/thaw cycles to liberate the adenovirus from the HEK293 cells. The crude adenovirus was then clarified using a 0.45 µm Steriflip®-HV device and mixed with 10X binding buffer. The viral mix was loaded onto the Fast-Trap™ Adenovirus Purification device and viral particles were bound to the membrane using vacuum filtration. After washing, the bound virus was eluted using an elution buffer. For concentration and buffer exchange, the eluted purified adenovirus was added to a 50 kDa NMWL Amicon® Ultra-4 filter unit along with exchange buffer of choice. The device was centrifuged and an additional volume of exchange buffer was added. After a final centrifugation step, the purified, concentrated adenovirus was collected and assessed for titer and purity.

Table 1.
Adenovirus purification results using Fast-Trap™ Adenovirus Purification and Concentration Kit and double CsCl gradient ultracentrifugation.

	Fast-Trap™ Adenovirus Purification and Concentration kit	Double CsCl Gradient
Amount crude adenovirus (ivp)	5.81×10^9	5.81×10^9
Amount purified adenovirus (ivp)	4.05×10^9	2.64×10^9
% virus recovery	70	45

Starting material (clarified adenovirus), flow-through (unbound material) and the final purified adenovirus were titered using the $TCID_{50}$ assay to determine the amount of viable virus particles that was bound and recovered. On average 90-100% of the adenoviral particles were bound to the membrane and more than 60% of the adenovirus was recovered in the elution. The whole purification workflow described above could be accomplished in about 30 minutes when low titer adenoviral samples were processed. Samples with higher titers (maximum capacity is approximately 1×10^{13} total adenoviral particles) could be processed in under two hours. Purity of eluted adenovirus samples was assessed using SDS-PAGE stained with Coomassie Blue (Figure 2). The majority of contaminating cellular and media proteins were removed (Figure 2, lane 1 and 2) and were not present in the elution fraction (Figure 2, lane 3).

The performance of the Fast-Trap™ Adenovirus Purification and Concentration Kit was compared to the traditional method of adenoviral purification, double cesium chloride (CsCl) gradient ultracentrifugation. Equal amounts of crude adenovirus were provided for each purification. Double CsCl gradient purification, processed by a specialized viral vector core facility, recovered 45% of the virus while EMD Millipore's Fast-Trap™ purification provided 70% virus recovery (Table 1).

Purity of the adenovirus samples was compared using SDS-PAGE (Figure 3A). This demonstrated that the Fast-Trap™ device (Figure 3A, lane 4) removes BSA and contaminating proteins comparable to a double CsCl purification (Figure 3A, lane 2). A Western blot with an anti-adenovirus serotype 5 polyclonal antibody (Abcam, Cat. No. 6982) confirmed that proteins detected in purified samples (Figure 3B, lane 2, and 4) are indeed adenoviral proteins.

Lentivirus Purification

Crude supernatant containing VTK-945, a VSV-G pseudotyped lentivirus expressing GFP, was provided by the UNC Vector Core. The virus was purified using the components of the EMD Millipore's Fast-Trap™ Lentivirus Purification and Concentration Kit.

A protocol similar to that described above for adenovirus purification was followed. The crude lentivirus supernatant was clarified using a 0.45 µm Steriflip®-HV device; clarified lentivirus was mixed with 10X binding buffer and purified using a Fast-Trap™ Lentivirus Purification device. For further concentration and buffer exchange of the eluted purified lentivirus sample, a 100 KDa NMWL Amicon® Ultra-4 filter unit was used.

To titer the different lentivirus fractions (starting material, flow-through, and purified sample), HEK293 cells were infected with serial dilutions of the virus. After three days, GFP positive cells were counted. The amounts of lentivirus bound to the membrane and recovered in elution were determined. Nearly 100% of the virus was bound, and recovery ranged from 40 to 70%. The entire workflow time including clarification, purification, and concentration/ buffer exchange ranged from 45 minutes for low titer samples to 90 minutes for high titer samples (maximum capacity of Fast-Trap™ Lentivirus Purification filter unit is approximately $2x10^8$ infectious viral particles). Purity was assessed by SDS-PAGE with Sypro® Ruby staining. It was confirmed that purification with the Fast-Trap™ device adequately removes contaminating proteins from crude lentiviral supernatant (Figure 4).

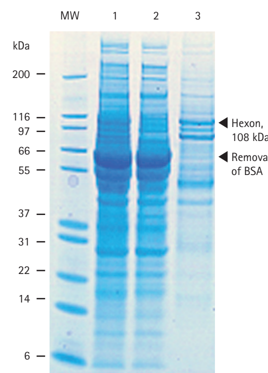
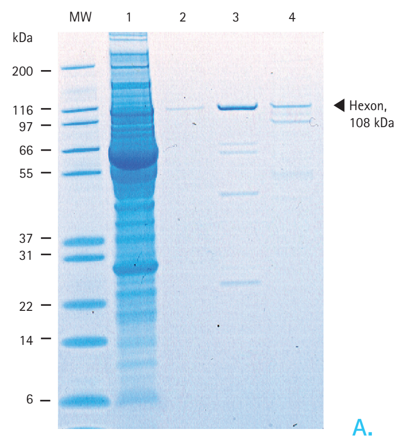


Figure 2.

SDS-PAGE stained with Coomassie Blue.

Equal volumes of crude clarified adenovirus (lane 1), flow-through fraction (lane 2) and eluate fraction (lane 3) of the Fast-Trap™ Adenovirus Purification device were loaded on a SDS gel and stained with Coomassie Blue. Adenoviral hexon protein was enriched and BSA was absent in the eluate fraction.



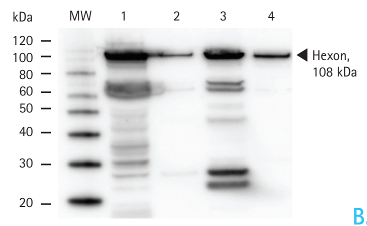


Figure 3.

Coomassie Blue stained SDS PAGE (3A) and Western blot analysis (3B) of adenovirus purification results.

0.3 µg protein loaded per lane, crude clarified adenovirus (lane 1), adenovirus purified with double CsCl gradient (lane 2), Ad5.CMV5-GFP viral stock from Q-Biogene (lane 3), adenovirus purified with EMD Millipore Fast-Trap™ Kit (lane 4). Presence of adenoviral proteins was confirmed by immunodetection with an anti-adenovirus serotype 5 polyclonal antibody.

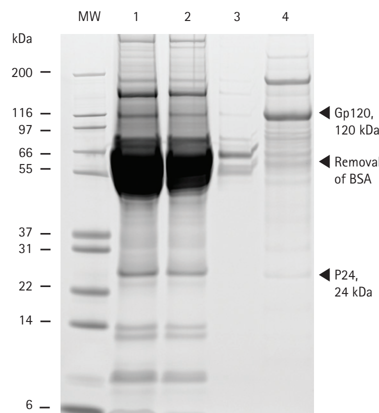


Figure 4.

SDS PAGE with Sypro Ruby staining.

Analysis of equal volumes of crude clarified lentivirus (lane 1), flow-through fraction (lane 2), wash fraction (lane 3) and eluate (lane 4) from the Fast-Trap™ Lentivirus Purification. Lentiviral Gp120 and p24 were detectable in the eluate and BSA was absent.

Performance of the Fast-Trap™ Lentivirus Purification and Concentration Kit was compared to the traditional method of lentiviral purification. Similar amounts of crude lentivirus were purified using the EMD Millipore kit and a sucrose gradient ultracentrifugation (outsourced to a viral vector core facility). The recovery of the virus by sucrose gradient purification was only about 22%.

Purity of the two samples was evaluated by SDS-PAGE with Sypro® Ruby staining. The load was normalized for infectious particles. Similar removal of contaminating proteins was demonstrated (Figure 5A). The presence of the 120 kDa band which corresponds to the lentiviral Gp120 glycosylated surface envelope protein in the Fast-Trap™ samples (Figure 5A, lane 2 and 3) can be explained by the different mechanism of purification. EMD Millipore Fast-Trap™ purification is based on binding of the viral particles onto a membrane which might explain the enrichment of surface envelope proteins in these samples. Western blot analysis with an anti-HIV p24 antibody (Cat. No. MAB8790) confirmed that proteins detected in purified samples (Figure 5B) are indeed lentiviral proteins.

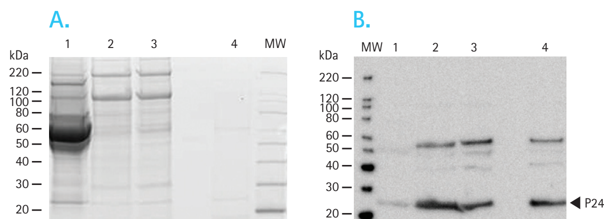


Figure 5.

Sypro Ruby stained SDS PAGE (5A) and Western blot analysis (5B) of lentiviral fractions.

3.4×10^5 ivp loaded per lane, crude clarified lentivirus (lane 1), eluate from Fast Trap™ Lentivirus Purification device (lane 2), Fast-Trap™ purified, concentrated and buffer exchanged lentivirus (lane 3), sucrose gradient purified lentivirus (lane 4). Presence of lentiviral proteins was confirmed by immunodetection with an anti-HIV p24 antibody.

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Summary

EMD Millipore's Fast-Trap™ Adenovirus and Lentivirus Purification and Concentration Kits provide a fast, safe, and easy alternative for viral purification. The kits contain the necessary components to accommodate the entire virus purification workflow. The purification results in high recovery of viable viral particles with good purity.

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