A New Vacuum-Based Method for Adenovirus Purification and Concentration

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Abstract

Adenovirus vectors have traditionally been purified away from cellular contaminants, expressed recombinant transgenes, and cell culture media serum proteins by cesium chloride or other similar density gradient based methods. However, these traditional methods present several disadvantages to the researcher. Density gradient techniques are lengthy, often requiring several days to complete. The process requires the use of ultracentrifuges which are expensive and not common equipment for the average laboratory. The technique for harvesting adenovirus bands from the density gradient after ultracentrifugation can also be cumbersome for many researchers.

We report here an improved method for the rapid purification and concentration of adenovirus serotype 5. This membrane-based method for purifying adenovirus is different from other similar membrane-based methods as it uses a much easier and safer vacuum-based device. Performance in terms of processing time, recovery, purity, and capacity is as good as or better than similar products currently available from other manufactures. The entire procedure including clarification of crude adenovirus, purification of adenovirus, buffer exchange, and concentration can be accomplished in under one hour and results in a concentrated, high titer, pure adenovirus in the buffer of choice.

Fast-Trap™ Adenovirus Purification and Concentration Kit Overview

The Fast-Trap Adenovirus Purification and Concentration Kit (Millipore #FTAV00003) contains all of the necessary reagents and devices to purify crude serotype 5 adenovirus and concentrate and/or perform buffer exchange of the final purified virus.

Clarification

Purification

Concentration

Sterilization

Step 1: Clarify crude adenovirus with Steriflip®-HV device.

Step 2: Dilute crude adenovirus with 10X binding buffer.

Step 3: Apply Equilibration buffer to Fast-Trap virus filter unit and vacuum filter.

Step 4: Apply diluted crude adenovirus from step 2 to Fast-Trap virus filter unit and vacuum filter.

Step 5: Apply Wash buffer to Fast-Trap virus filter unit and vacuum filter.

Step 6: Apply Elution Buffer to Fast-Trap virus filter unit and vacuum filter.

Step 7: Incubate for 5 minutes.

Step 8: Vacuum filter remaining Elution buffer to elute virus.

Step 9 (Optional): Repeat steps 6-8.

Step 10: Transfer eluted virus and exchange buffer to Amicon® Ultra filter unit and centrifuge.

Step 11: Add exchange buffer to Amicon Ultra filter unit and centrifuge.

Step 12: Recover virus sample from filter unit.

Step 13 (Optional): Apply concentrated virus to Ultrafree®-MC filter and centrifuge. Recover filter-sterilized sample.

Processing Time

Figure 1.

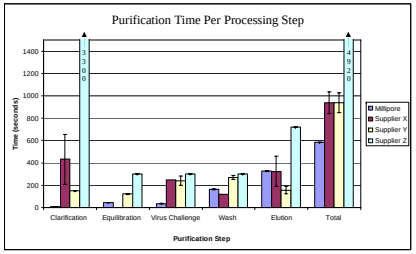

Table 1.

Manufacturer	Format	Average Processing Time					
		Clarification	Equilibration	Virus Challenge	Wash	Elution	Total Time
Millipore	Vaccuum	10 sec.	45 sec.	37 sec.	2 min. 43 sec.	5 min. 28 sec.	9 min. 42 sec.
Supplier X	Syringe Filter	7 min. 12 sec.	n/a	4 min. 6 sec.	2 min.	5 min. 25 sec.	15 min. 40 sec.
Supplier Y	Syringe Filter	2 min. 30 sec.	2 min. 3 sec.	4 min.	4 min. 30 sec.	2 min. 37 sec.	15 min. 40 sec.
Supplier Z	Centrifugal	55 min. (clogging issues)	5 min.	5 min.	5 min.	12 min.	82 min.
-	CsCl Density Gradient	-	-	-	-	-	48-72 hours

Figure 1. / Table 1. Average processing times for Millipore Fast-Trap adenovirus purification kit compared to 3 other commercially available adenovirus purification kits. Two devices were tested from each manufacturer and were all challenged with equal amounts of adenovirus from the same preparation (1.5 x 10^{10} infectious adenovirus particles). ***Millipore's Fast-Trap Adenovirus Purification Kit required less time to complete purification than other similar adenovirus purification kits.***

Buffer Exchange & Concentration

Millipore's Fast-Trap Adenovirus Purification Kit provides Amicon Ultra-4 devices with a 50 kDa NMWCO for both concentration and buffer exchange of purified eluate. Optimal recovery of infectious adenovirus particles is achieved when retentate volume is kept at or above 500 µL throughout the concentration and buffer exchange process. Reduction of retentate volume below 500 µL may lead to loss of virus infectivity due to aggregation of adenovirus particles.

Figure 2.

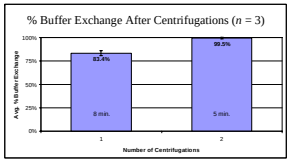

Figure 2. Percent buffer exchange as measured after a single centrifugation or two buffer exchanges and centrifugation cycles (1,500 x g). In this experiment 1.5 mL Fast-Trap elution buffer was combined with 2.5 mL exchange buffer (250 mM NaCl) for a total of 4 mL in the Amicon Ultra device. As indicated by the graph, 83.4% of elution was exchanged after the first centrifugation and 99.5% of elution buffer was exchanged following a second centrifugation. ***Thus, Buffer exchange using Amicon Ultra-4 device with 50 kDa NMWCO is a fast and effective method for buffer exchange following Fast-Trap purification.***

Recovery of Infectious Adenovirus Particles

Figure 3.

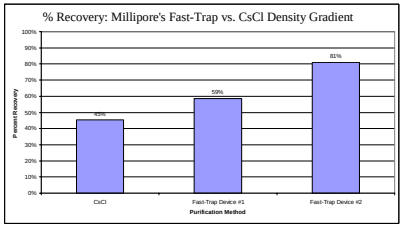

Figure 3. 50% Tissue Culture Infectious Dose ($TCID_{50}$) data comparing recovery of infectious adenovirus particles using the traditional cesium chloride (CsCl) density gradient purification to Millipore's Fast-Trap purification method. 5.81 x 10^9 infectious adenovirus particles were purified using either traditional CsCl or two Fast-Trap purification devices. ***Using the Fast-Trap method, an average 70% of infectious adenovirus particles were recovered compared to 45% using the traditional density gradient method.***

Figure 4.

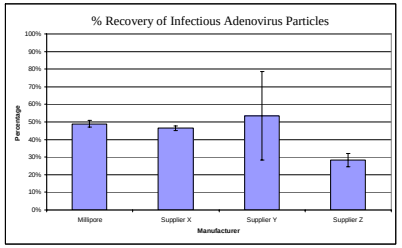

Figure 4. Comparison of Millipore's Fast-Trap Adenovirus Purification Kit to three similar commercially available adenovirus purification kits. Two adenovirus purification devices from each kit were challenged with 1.5 x 10^{10} infectious virus particles (as measured by $TCID_{50}$) and purification was carried out following the protocol provided by each supplier. ***Recovery of infectious adenovirus particles shows Millipore's Fast-Trap kit performs as good as or better than other similar competitive adenovirus purification kits.***

Total Adenovirus Particle Capacity

Table 2.

Manufacturer	Kit	Capacity
Millipore	Fast-Trap Adenovirus Purification Kit	1 x 10^{13}
Supplier X	Adenovirus Purification Kit	1 x 10^{13}
Supplier Y	Adenovirus Purification Kit	1 x 10^{12}
Supplier Z	Adenovirus Purification Kit	1 x 10^{13}

Table 2. Capacity for total adenovirus particles claimed for similar commercially available adenovirus purification kits. ***In comparison to these kits, Millipore's Fast-Trap Adenovirus Purification Kit demonstrates similar or higher capacity for recoverable adenovirus particles.***

Purity

Figure 5.

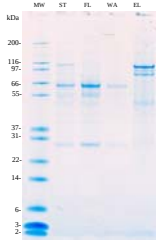

Figure 5. SDS-PAGE stained with Coomassie Blue loaded with 1.3 µg/lane total protein. MW = Molecular weight marker, ST = Crude starting material, FL = Fast-Trap flow-through, WA = Fast-Trap wash fraction , EL = Fast-Trap elution fraction. ***Results indicate the majority of contaminating proteins in the crude starting material flow directly into the filtrate and do not bind to the membrane. The wash buffer effectively removes any weakly bound proteins from the membrane, leaving highly purified adenovirus in the elution fraction.***

Figure 6.

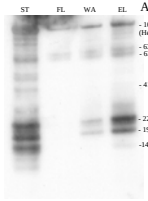

Figure 6. Western blotting results using Abcam (Ab6982) anti-adenovirus polyclonal primary antibody on SNAP i.d.™ Protein Detection System (Millipore #WBAVDBASE) with 0.9 µg total protein per lane. ST = Crude starting material, FL = Fast-Trap flow-through, WA = Fast-Trap wash fraction, EL = Fast-Trap elution fraction. ***Results confirm Fast-Trap elution fraction proteins are adenovirus proteins.***

Figure 7.

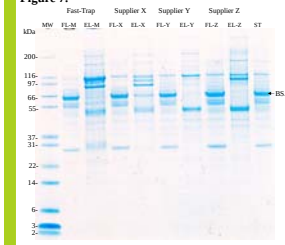

Figure 7. SDS-PAGE stained with Coomassie Blue loaded with 2.6 µg/lane total protein. MW = Molecular weight marker, FL-M = Fast-Trap flow-through, EL-M = Fast-Trap elution fraction, FL-X = Supplier X flow-through, EL-X = Supplier X elution fraction, FL-Y = Supplier Y flow-through, EL-Y = Supplier Y elution fraction, FL-Z = Supplier Z flow-through, EL-Z = Supplier Z elution fraction, ST = Crude adenovirus starting material. ***Results indicate Millipore's Fast-Trap Adenovirus Purification Kit binds more adenovirus hexon protein (108 kDa) than similar commercially available kits as demonstrated by the lack of hexon protein band in the flow-through compared to the competitor flow-through fractions. Comparison of elution fractions demonstrates effective removal of contaminating bovine serum albumin (BSA) and other cell culture proteins.***

Conclusions

Millipore's new vacuum-based method for adenovirus purification and concentration successfully addresses several problems associated with common purification techniques such as density gradient centrifugation and other membrane-based virus purification methods.

The Fast-Trap Adenovirus Purification and Concentration Kit offers:

- Improved ease-of-use and handling over traditional syringe-based purification methods and density gradient purification methods
- Faster processing times than many other available adenovirus purification kits
- High capacity for adenovirus particles that is as good or better than kits from other suppliers
- Purity of eluted adenovirus that is similar to other adenovirus purification kits
- Recovery of infectious adenovirus particles that is superior to traditional CsCl based methods and is as good or better than other membrane-based purification methods
- Efficient concentration and buffer exchange of the purified adenovirus sample into the exchange buffer of choice