

Mega-scale adenovirus purification system

Our Adeno-X Mega Purification Kit allows you to purify large amounts of recombinant adenovirus in 1.5 hours or less. With a simple protocol and a powerful syringe pump, you can purify up to 10^{13} viral particles without affecting infectivity. Adeno-X purification achieves nearly complete recovery of the infectious particles present in your crude supernatant, and the purified stocks are stable for years at -70°C . The kit is excellent for any application requiring mega-scale amounts of purified adenovirus, starting from cultures of twenty-five 15-cm dishes.

- Easy-to-use, streamlined protocol
- Chromatographic method eliminates messy CsCl gradients
- No more handling of large culture volumes

Introduction ^

Large-scale simplicity

The kit includes two filter assemblies and all the buffers needed to make two mega preps. All you need is a reliable syringe pump to provide automatic and consistent flow rate control so that your prep works every time (we recommend the GENIE Plus syringe pump from Kent Scientific). The straightforward protocol allows you to purify the adenovirus directly from the cell pellet, eliminating time-consuming filtration of the culture supernatant, which contains only small amounts of virus (~2% of that found in the pellet). You simply pellet the cells when the cytopathic effect (CPE) is nearly complete, lyse the cells, and purify the virus.

Protocol overview ^

Virus chromatography is quicker & easier than CsCl gradients

As with all of our Adeno-X purification kits, the virus is purified chromatographically, using a unique membrane that selectively binds adenoviral particles. Unlike complicated CsCl gradients, chromatographic purification is safe, nontoxic, and requires no advanced training or expensive ultracentrifugation equipment. The virus-binding membranes are housed in a single-use, multi-membrane cartridge assembly that fits securely on a standard size BD Luer-Lok syringe. For added convenience, the purification assembly comes preassembled, sterile, and ready to use.

A convenient one-way valve makes purification easier by eliminating the need to disconnect the syringe for refilling during the binding and washing steps. Liquids are brought into the syringe through the one-way valve merely by withdrawing the plunger. Depressing the plunger drives the solutions through the purification cartridge—it couldn't be easier!

All you need is a powerful pump to make the procedure rapid and easy

From beginning to end, the entire procedure is very fast and straightforward (Figure 1). Once the CPE is nearly complete in your amplification culture, you harvest and lyse the cells (to release the virus) and prepare the lysate for purification. To collect the virus, you can use a syringe pump to drive the clarified lysate through the three-tiered purification cartridge. The pump provides consistent pressure and flow rates to foster more efficient virus binding and excellent virus recovery, and eliminates the need to control the flow by hand. Bound virus particles are washed once and then eluted from the membrane using a convenient volume of elution buffer. The streamlined protocol, from harvest to elution, takes just 1–1.5 hours to complete.

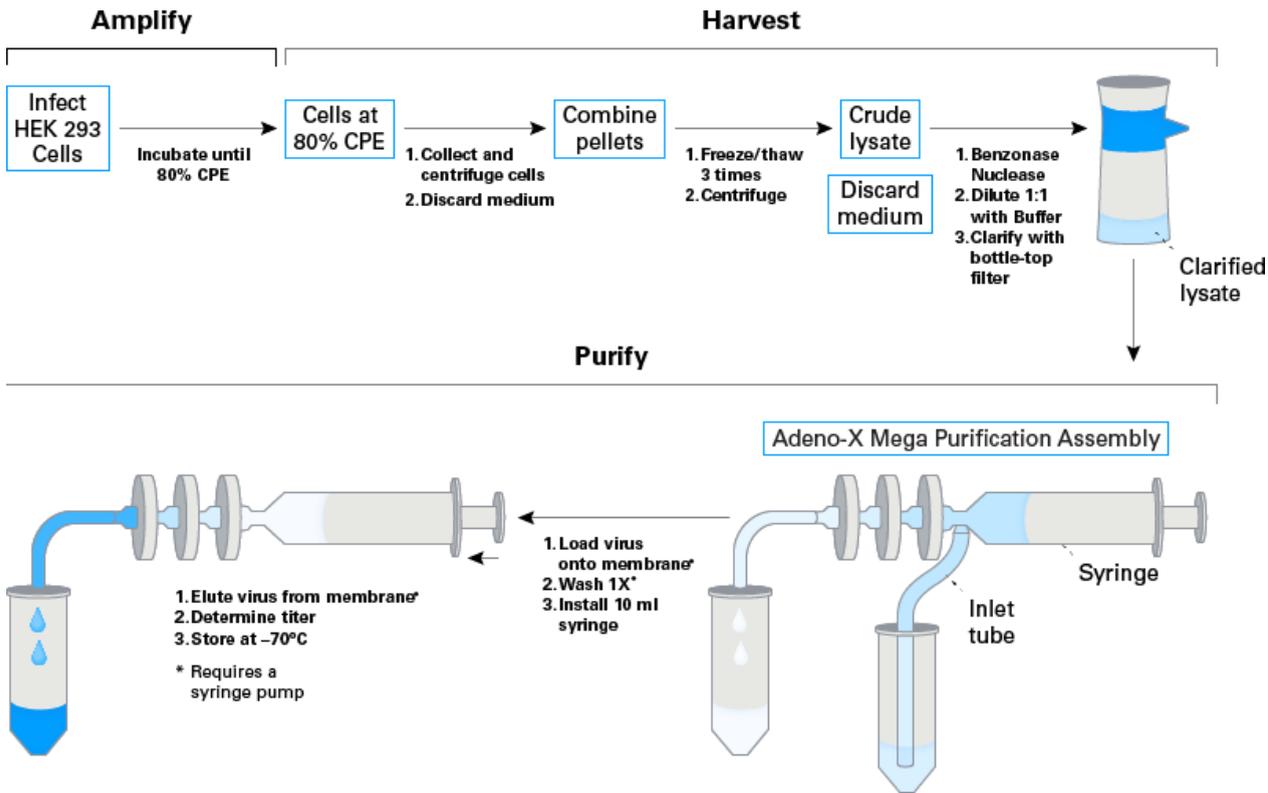


Figure 1. Adeno-X Mega Purification Kit protocol. From a large-scale culture of infected HEK 293 cells (~25, 15-cm plates), cells are collected and lysed by multiple freeze-thaw cycles. Nucleic acids are digested to reduce viscosity, then the lysate is clarified and passed through the purification filter assembly. After a brief wash, purified virus is eluted and ready to be used, titered, or stored. The entire process takes less than 1.5 hr.

Results

Achieve high titers of high quality virus

The titer and purity of virus produced by our Adeno-X purification kits are comparable to those achieved by CsCl density gradient centrifugation. The purified virus yields are characterized by excellent recoveries and high infectivity (Figure 2). In adenovirus preparations, not all particles are completely formed and infectious. With Adeno-X purification kits, ratios of virus particles to infectious units (IFU) can be as low as 20:1. Depending on the adenoviral backbone used and the nature of the expression cassette, the Adeno-X mega kit can yield up to 10^{13} adenoviral particles, which potentially corresponds to as much as 5×10^{11} IFU. A DsRed-Express ViraTrak virus purified with Adeno-X technology efficiently infects HEK 293 cells, which undergo a timely CPE and produce high levels of DsRed-Express protein (Figure 3).

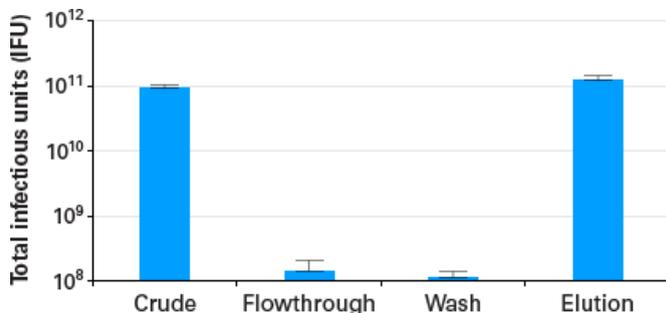


Figure 2. Adeno-X Mega purification yields nearly quantitative recoveries. HEK 293 cells were seeded and infected with Adeno-X ViraTrak DsRed-Express virus, and harvested when the cytopathic effect was nearly complete. The resulting adenovirus was then purified according to the Adeno-X Mega Purification Kit protocol. Total infectious units (IFU) \pm standard deviation for each fraction (crude, flowthrough, wash, and eluate), were determined by infecting fresh HEK 293 cells with dilutions of each fraction, and counting the fluorescent cells under a fluorescence microscope 48 hr later. Virtually all of the starting material was recovered in the eluate as purified virus. Purified virus demonstrated greater apparent infectivity than the virus present in the crude lysate. The process was efficient and fast, allowing 1.23×10^{11} adenoviral IFU to be purified in 1.5 hour.

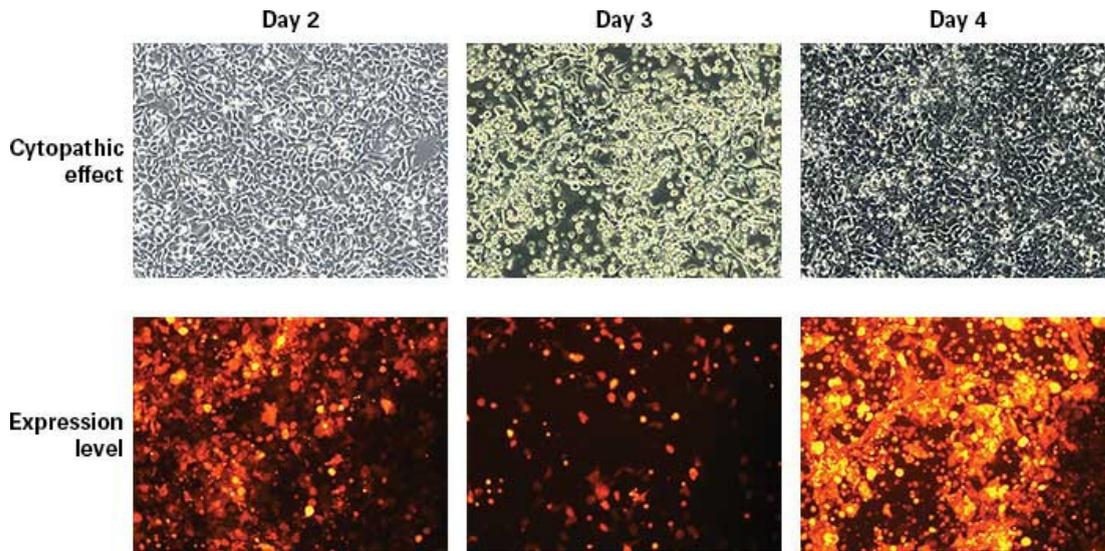


Figure 3. Progression of the CPE and fluorescent protein expression in infected HEK 293 cells. HEK 293 cells seeded on a 6-well plate were transduced with an Adeno-X ViraTrak DsRed-Express adenovirus at an MOI of ~1–2. During the following 4 days, the CPE (top row) and DsRedExpress expression (bottom row) were monitored by phase contrast and fluorescence microscopy, respectively. By day 4, the CPE was nearly complete and correlated with high levels of DsRed-Express protein.

Conclusions

All of our Adeno-X purification kits are characterized by outstanding virus recovery, and produce highly purified and highly infectious adenovirus with low viral particle/IFU ratios. The Adeno-X Mega Purification Kit simplifies adenoviral purification and provides large-scale convenience at a great price.



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Cat. #	Product	Size	License	Quantity	Details
631032	Adeno-X™ Mega Purification Kit	2 Preps		*	↑
<p>The Adeno-X Mega Purification Kit is a chromatography-based purification system for concentrating and purifying infectious adenovirus. The kit provides all of the buffers and filters needed to purify adenovirus from cell lysate. This Kit should be used with a continuous flow syringe pump (see User Manual for details).</p> <p>↓</p>					
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Takara Bio USA, Inc.

United States/Canada: +1.800.662.2566 • Asia Pacific: +1.650.919.7300 • Europe: +33.(0)1.3904.6880 • Japan: +81.(0)77.565.6999

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