Adeno-X™
Virus Purification Kits
User Manual

Cat. Nos. 631532
631533,
631534
PT3680-1 (PR5X1126)
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Note: The viral supernatants produced by transfecting HEK 293 cells with recombinant Adeno-X Viral DNA could, depending on your DNA insert, contain potentially hazardous recombinant virus. Due caution must be exercised in the production and handling of recombinant adenovirus. **The user is strongly advised not to create adenoviruses capable of expressing known oncogenes**.

Appropriate NIH, regional, and institutional guidelines apply, as well as guidelines specific to other countries. NIH guidelines require that adenoviral production and transduction be performed in a Biosafety Level 2 facility. For more information, see appropriate HHS publications. A link to the *NIH Guide to Biosafety in Microbiological and Biomedical Laboratories* can be found at **www.clontech.com/clontech/expression/adeno**. Section IV in this User Manual contains a brief description of Biosafety Level 2 as well as other general information and precautions.

I. Introduction & Protocol Overview

The **Adeno-X™ Virus Purification Kit** is a complete filtration-based system for purifying and concentrating recombinant adenovirus. It provides a superior alternative to cesium chloride (CsCl) density gradient centrifugation. Although centrifugation in CsCl is an extremely effective method for purifying adenovirus, it is also time-consuming and technically demanding. Furthermore, the procedure is restrictive, for it is not easily scaled up or down. A broader range of volumes can be purified with the Virus Purification Kit (Table I), so you can collect recombinant adenovirus after the cytopathic effect is complete—when the viral titer is higher. The Adeno-X Purification Kit is not only faster, but also easier and safer than CsCl methods. Results show it is just as effective (Table I).

A chromatographic method

The Adeno-X Virus Purification Kit lets you purify adenovirus chromatographically, using an adsorbent membrane that selectively binds adenoviral particles based on their distinctive surface-associated properties. The membrane is housed in a small, single-use cartridge, which fits on disposable Luer-Lok™ syringes or house vacuum lines. As virus-containing medium is drawn through the cartridge, adenoviral particles are trapped and effectively removed from the solution (Figure 1). Once bound, the viral particles can then be eluted with a small volume of 1X Elution Buffer (provided).

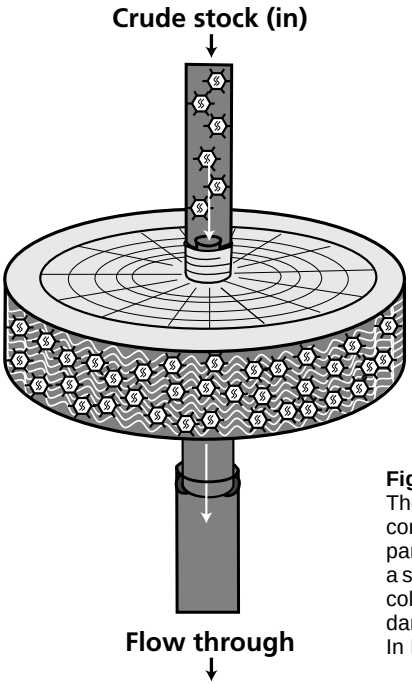

Figure 1. Principle of Adeno-X™ Virus Purification. The Adeno-X Purification Filter, developed by VIRAPUR, consists of several filters that selectively bind adenoviral particles as they pass through. The filters are enclosed in a small cylindrical cartridge, which resembles a radial flow column, complete with entrance and exit ports. In standard-size kits, the cartridge is 3.5 cm wide and 1 cm thick. In Mega Kits, it is 6.5 cm wide and 1 cm thick.

I. Introduction & Protocol Overview *continued*

The purification apparatus

The Adeno-X Purification Kit consists of four main parts: the holding vessel, the Tubing Assembly, the Purification Filter, and the vacuum source (Figure 2). The holding vessel contains the adenoviral stock to be purified. For standard size purifications, the holding vessel is usually the bottom half of a Bottle-Top Filter Unit (provided); larger vessels (1 L vessels) must be used for Mega Kits. In standard Kits, the Tubing Assembly, Purification Filter, and vacuum source (60 ml syringe) are all pre-assembled and referred to as the Syringe-Filter Assembly. In Mega Kits, the Tubing Assembly consists of two subparts, labeled A and B. Tubing Assembly A will connect the holding vessel to the Purification Filter, while Tubing Assembly B will connect the Purification Filter to your house vacuum line. Note that the tubing assemblies are already fitted with male and female Luer-Lok™ adaptors to connect the tubing to the ports on the Purification FIlter. Tubing Assembly B also contains a two-way adaptor for connection with a house vacuum line. In addition, the Mega Kit Tubing Assembly contains a prepositioned Pinch Clamp, which is used to help control the rate of flow through the filter and to prevents backflow when the vacuum is turned off.

The Adeno-X Purification Filter (developed by VIRAPUR) is the central part of the assembly. In standard kits, the filter connects via the one-way valve to the Luer-Lok™ tip on a plastic disposable syringe—the vacuum source (Figure 2, Panel A). In Mega Kits, the Filter connects to a house vacuum line via Tubing Assembly B (Figure 2, Panel B). The Purification Filter has no directionality, but in assembling the apparatus you will find that the Filter can be connected in only one direction because of the male and female fittings.

The purification protocol

The Adeno-X Purification process consists of three main steps: amplifying adenovirus, harvesting adenovirus, and purifying adenovirus (Figure 3). Amplification takes place in HEK 293 cell cultures. The cultures are first infected with an adenoviral stock, and then incubated until cytopathic effects are complete. Adenovirus is then harvested by freezing and then rapidly thawing infected cells. After centrifugation, the amplified stock is further clarified by filtration with a Bottle-Top Filter (provided).

In the final step, the filter-clarifed solution is combined with an equal volume of 1X Dilution Buffer, and then drawn through the Adeno-X Purification Filter, assembled as shown in Figure 2. After the entire volume has been filtered, the filter assembly is rinsed with 1X Wash Buffer. The trapped adenovirus is then eluted from the filter cartridge with a small volume of 1X Elution Buffer. The purified, high-titer adenoviral stock can be cryogenically preserved in 1X Elution Buffer or concentrated and stored in 1X Formulation Buffer. Store your aliquots at –70°C. All these steps must be conducted under sterile conditions in a Biosafety Level 2 hood.

I. Introduction & Protocol Overview *continued*

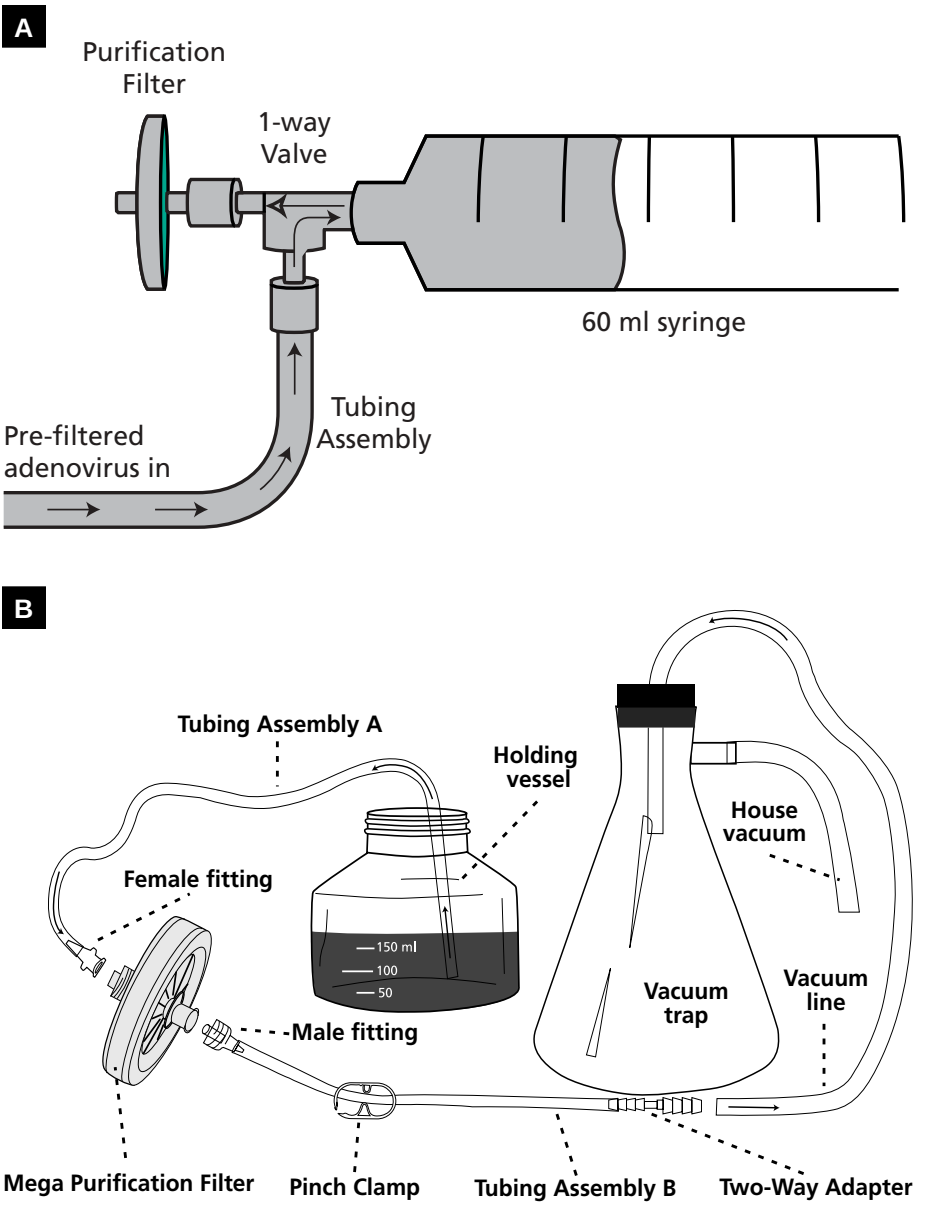

Figure 2. The Adeno-X™ Virus Purification Assembly. Both the Standard (**Panel A**) and Mega (**Panel B**) assemblies are shown. The apparatus should be assembled under sterile conditions in a Biosafety Level 2 hood.

I. Introduction & Protocol Overview *continued*

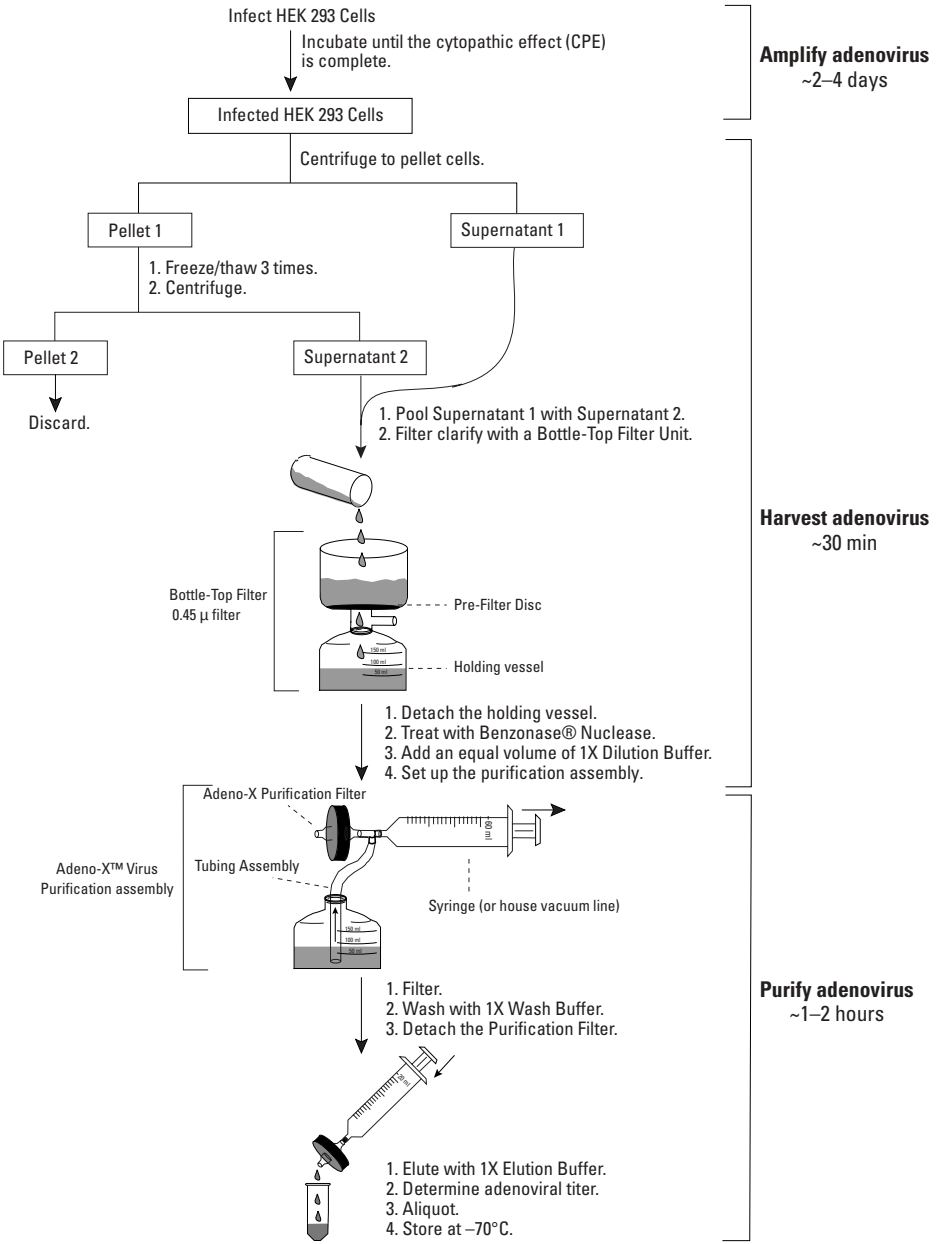

Figure 3. Overview of the Adeno-X™ Virus Purification Protocol. The illustrations shown correspond to the apparatus and procedures used in standard-size Purification Kits. For the Mega apparatus, refer to Figure 2, or PT3767-2.

I. Introduction & Protocol Overview *continued*

Advantages of the Adeno-X™ Virus Purification Kit

Purifying adenovirus with the Adeno-X Kit takes significantly less time and labor than CsCl density gradient centrifugation. CsCl methods, though effective, require meticulous preparation and may take as long as 3 days to complete (Graham & Prevec, 1991). With the Adeno-X method, however, once your stocks have been amplified, they can be processed in less than 2 hours. Both standard- and Mega-scale kits are available, and each kit has a wide range of viral capacities, so you can scale up or down without difficulty. Because our method lets you harvest adenovirus from both the growth medium and the cell pellet, you obtain consistently high yields of adenovirus without having to worry about when to pull the cultures from the incubator. Simply remove cells when the cytopathic effect (CPE) is complete and harvest.

Purity comparable to that achieved with CsCl gradients

In addition to its time and cost-cutting benefits, the Adeno-X Virus Purification Kit is very effective, producing chromatographically pure adenovirus that is suitable for both *in vitro* and *in vivo* studies. In fact, the titer and purity of the adenovirus is comparable to that obtained with CsCl gradient centrifugation (Table I and Figure 4).

TABLE I. PURIFICATION OF RECOMBINANT ADENOVIRUS

Purification method	Final volume	OD_{260} Titer[a] (total particles)	Infectious Titer[b] (infectious units)	Particle/infectious virus[c]
Adeno-X[d]	2.5 ml	3.87×10^{11}	1.72×10^{10}	22.5
CsCl[e]	2.1 ml	2.62×10^{11}	6.85×10^{9}	38.2

[a] OD_{260} measurements provide an estimate of the total number of viral particles, both infectious and noninfectious.

[b] Measured with the Adeno-X Rapid Titer Kit (Cat. No. 631028).

[c] This ratio, which varies among viral stocks, typically falls between 25:1 and 100:1.

[d] The values shown are the mean of 6 standard purifications.

[e] The values shown are the means of 3 purifications.

I. Introduction & Protocol Overview *continued*

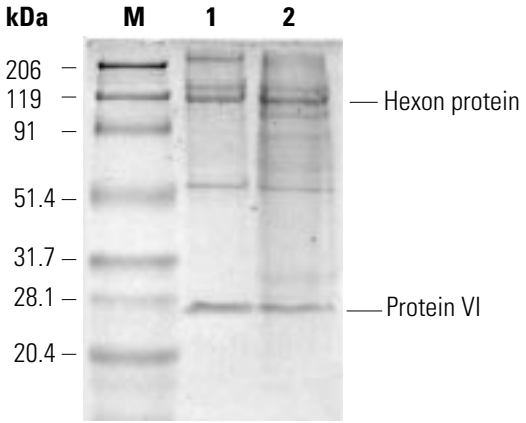

Figure 4. SDS-PAGE analysis of purified adenovirus. Adenoviral samples were first dialyzed against 10 mM Tris-HCl, pH 7.4; 7% sucrose, and then resolved by electrophoresis on an SDS/10% polyacrylamide gel. The gel shown was stained with Coomassie Brilliant Blue R250. Lane 1 = adenovirus purified by the Adeno-X method; Lane 2 = adenovirus purified by CsCl density gradient centrifugation; Lane M = molecular weight markers. Lanes 1 and 2 were loaded with 14 µg of protein.

II. List of Components

Store Benzonase® Nuclease at –20°C. Store all other components at room temperature.

The **Adeno-X™ Virus Purification Kits** (Cat. Nos. 631532 & 631533) contain sufficient reagents for 1 and 5 standard purifications, respectively.

Cat. No. 631532	Cat. No. 631533	
• 20 ml	100 ml	5X Dilution Buffer[a]
• 12 ml	60 ml	5X Wash Buffer[a]
• 5 ml	25 ml	1X Elution Buffer[a]
• 5 ml	15 ml	1X Formulation Buffer[b]
• 40 µl	200 µl	Benzonase® Nuclease (25 U/µl)
• 1	5	Bottle-Top Filter
• 1	5	Pre-Filter Disc
• 1	5	Adeno-X™ Syringe-Filter Assembly
• 1	5	Syringe (5 ml)

The **Adeno-X™ Virus Purification Mega Kit** (Cat. No. 631534) contains sufficient reagents for 1 mega purification.

Cat. No. 631534	
• 120 ml	5X Dilution Buffer[a]
• 100 ml	5X Wash Buffer[a]
• 30 ml	1X Elution Buffer[a]
• 30 ml	1X Formulation Buffer[b]
• 200 µl	Benzonase® Nuclease (25 U/µl)
• 2	Bottle-Top Filters
• 2	Pre-Filter Discs
• 1	Tubing Assembly A
• 1	Tubing Assembly B
• 1 Mega	Adeno-X™ Purification Filter
• 1	Syringe (20 ml)

[a] The compositions of the Dilution, Wash, and Elution Buffers are trade secrets of VIRAPUR.

[b] 1X Formulation Buffer consists of 2.5% glycerol (w/v); 25 mM NaCl; and 20 mM Tris-HCl, pH 8.0 (GTS buffer; Hoganson, *et al.,* 2002).

III. Additional Materials Required

The following materials are required but not supplied.

- **100 mM Tris-HCl, pH 7.4**
- **Phosphate Buffered Saline** (PBS)
- **Tissue culture plates and flasks**
  (e.g., 10 or 15 cm plates, T75 or T175 flasks, or roller bottles)
- **Centrifuge**
  (Swinging-bucket and fixed-angle rotors compatible with 15 ml, 50 ml, and if needed, 100 ml centrifuge tubes)
- **Sterile 50 ml and 250 ml centrifuge tubes**
  (For small- and large-scale purifications, respectively)
- **Biosafety hood with vacuum source**
- [Optional: instead of Benzonase® Nuclease] **Deoxyribonuclease I from bovine pancreas** (Sigma Cat. No. D4513)
- **Centriprep YM-50 Centrifugal Filter Unit**
  (Millipore Corporation, Cat. Nos. 4310, 4311, or 4323; 50,000 NMWL)

  These units, which hold 15 ml, are best for concentrating adenovirus purified with our Mega Kits.
- **Centricon YM-50 Centrifugal Filter Unit**
  (Millipore Corporation, Cat. Nos. 4243, 4224, or 4225; 50,000 NMWL)

  These units, which hold 2 ml, are best for concentrating adenovirus purified with our Standard Kits.

IV. Safety & Handling of Adenoviruses

The protocols in this User Manual require the production, handling, and storage of infectious adenovirus. It is imperative to fully understand the potential hazards of and necessary precautions for the laboratory use of adenoviruses.

The National Institute of Health and Center for Disease Control have designated adenoviruses as Level 2 biological agents. This distinction requires the maintenance of a Biosafety Level 2 facility for work involving this virus and others like it. The virus packaged by transfecting HEK 293 cells with the adenoviral-based vectors described here are capable of infecting human cells. These viral supernatants could, depending on your gene insert, contain potentially hazardous recombinant virus. Similar vectors have been approved for human gene therapy trials, attesting to their potential ability to express genes *in vivo*.

For these reasons, due caution must be exercised in the production and handling of any recombinant adenovirus. **The user is strongly advised not to create adenoviruses capable of expressing known oncogenes.**

For more information on Biosafety Level 2, see the following reference:

- *Biosafety in Microbiological and Biomedical Laboratories*, 4th Edition (May 1999) U.S. Department of Health and Human Services. (Available at http://bmbl.od.nih.gov.)

Biosafety Level 2:
The following information is a brief description of Biosafety Level 2. *It is neither detailed nor complete*. Details of the practices, safety equipment, and facilities required for Biosafety Level 2 are available in the above publication. If possible, observe and learn the practices described below from someone who has experience working with adenoviruses.

- Practices:
  - perform work in a limited access area
  - post biohazard warning signs
  - avoid generating aerosols
  - decontaminate potentially infectious wastes before disposal
  - take precautions with sharps (e.g., syringes, blades)
- Safety equipment:
  - biological safety cabinet, preferably Class II (i.e., a laminar flow hood with microfilter [HEPA filter] that prevents release of aerosols)
  - protective laboratory coats, face protection, double gloves
- Facilities:
  - autoclave for decontamination of wastes
  - unrecirculated exhaust air
  - chemical disinfectants available for spills

V. Adenovirus Purification Protocol

PLEASE READ ENTIRE PROTOCOL BEFORE STARTING.

The following protocol describes how to amplify, harvest, and purify adenovirus. Directions for both standard- and mega-scale purifications are given. If you are performing a mega purification, use the volumes and quantities given in brackets.

A. Amplify Adenovirus in HEK 293 Cells

1. Infect low-passage HEK 293 cells with your adenovirus stock. For general infection and culture parameters, refer to Table II.

   Notes:

   - You may infect HEK 293 cells while they are in suspension or after they have adhered to the plate. We generally infect in suspension. For example, to amplify adenovirus for a standard purification, we infect 1.25 x 10^8 cells with 3.75 x 10^8 ifu of adenovirus. The components are first mixed in a sterile tube or flask in 100 ml of growth medium. The mixture is then evenly distributed among five 15 cm culture plates (2.5 x 10^7 cells/plate). Cytopathic effect is usually complete after 2–4 days of incubation. For mega purifications, we infect 6.25 x 10^8 cells with ~2 x 10^9 ifu of adenovirus and seed twenty-five 15 cm plates.
   - Any type of culture vessel may be used—e.g., 10 cm or 15 cm plates, T75 or T175 flasks, or roller bottles.
   - Your adenoviral stock may be in the form of a crude cell lysate or a purified viral preparation. If you are using a crude stock, and the recombinant adenovirus encodes a potentially cytotoxic protein, you may need to purify the stock first before performing additional amplifications.
   - If you wish to infect adherent HEK 293 cells, the cell monolayer should be 50–90% confluent at the time of infection.

2. Incubate infected cells until cytopathic effects are complete (~2–7 days).

   Note: Infected cells typically remain intact, but round up and may detach from the plate. Infected cells may detach individually or in "grape-like" clusters that float in the medium. These morphological changes are collectively referred to as the cytopathic effect (CPE).

TABLE II. GUIDELINES FOR ADENOVIRUS AMPLIFICATION AND PURIFICATION

Purification Size	Cat. No.	Optimum number of infected cells[a]	Optimum M.O.I.[b]	Maximum allowable culture volume (ml)[c]
Standard	631532, 631533	1–2 x 10^8	2–5	100
Mega	631534	5–10 x 10^8	2–5	500

[a] The number of infected cells present in culture when adenovirus is harvested.

[b] M.O.I. = multipicity of infection. The M.O.I. is expressed numerically as the number of infectious units (ifu) or plaque forming units (pfu) per cell.

[c] The maximum volume that may be processed for purification using one filtration unit.

V. Adenovirus Purification Protocol *continued*

B. Harvest Adenovirus (See Figure 3, Page 6 for procedural diagram)

1. Using a sterile pipette, transfer the cells to one or more sterile conical centrifuge tubes.

   Important: Do not wash or trypsinize cells. Transfer the growth medium directly from the culture vessels to the centrifuge tube. Infected cells that still adhere to the bottom or sides of the culture plate can be dislodged by gentle agitation or by pipetting up and down.
2. Centrifuge at ~1,500 x g for 5 min in a swinging-bucket centrifuge.
3. Collect and save the supernatant (Supernatant 1) in a sterile centrifuge tube. Store Supernatant 1 at 4°C until use (Step 8).
4. Resuspend the pellet (Pellet 1) in 5 ml [25 ml] of Supernatant 1 or in 5 ml [25 ml] sterile 100 mM Tris-HCl, pH 7.4.

   Do not use PBS, as it inhibits some endonucleases. Endonuclease is used below to remove contaminating cellular nucleic acids from filter-clarified cell lysate.
5. Lyse cells with three consecutive freeze-thaw cycles: Freeze cells in a dry ice/ethanol bath; thaw cells by placing the tube in a 37°C water bath. Mix cells by vortexing after each thaw. Additional freeze-thaw cycles will be detrimental to adenovirus particles. Therefore, **do not exceed three freeze-thaw cycles.**
6. After the third cycle, centrifuge at 1,500 x g for 5 min to pellet the debris.
7. Collect and save the supernatant (Supernatant 2) in a sterile centrifuge tube. Discard the pellet (Pellet 2).
8. Pool Supernatant 1 (from Step 3) with Supernatant 2 (from Step 7).
9. Unwrap a Bottle-Top Filter Unit and place it in the biosafety hood.

   Note: If you are performing a mega purification, you will need two Bottle-Top Filter Units and a sterile 1-L bottle or T175 flask to hold the filtrate.
10. Remove the lid from the top of the unit and carefully place the Pre-filter Disc on top of the 0.45 micron filter.
11. Connect the Bottle-Top Filter Unit to a vacuum source.
12. With the vacuum off, carefully pre-wet the Pre-Filter with 5–10 ml of pooled lysate or medium from Step 8, then add the remainder to the top of the bottle-top filter unit. Be careful not to dislodge the Pre-Filter. Do not overfill.
14. Turn on the vacuum. When the bottom collection vessel becomes full, disconnect the vacuum line, and unscrew the collection vessel. Save the filtrate in the sterile filter unit bottle.

V. Adenovirus Purification Protocol *continued*

Mega purifications: If the first Bottle-Top Filter Unit becomes clogged (i.e., if the flow decreases to a drip), switch to the second Bottle-Top Filter Unit.

15. Remove contaminating cellular DNA by adding Benzonase® Nuclease to the filtrate (40 µl for standard prep.; 200 µl for Mega prep.). The final concentration should be 10 units Benzonase® Nuclease/ml. Incubate at 37°C for exactly 30 min.

    Notes

    Nuclease treatment decreases the viscosity of the solution so that it can be more easily drawn through the Adeno-X Purification Filter (below). By degrading cellular DNA, nuclease treatment also eliminates a potential contaminant, one that is known to interfere with the OD_{260} method of adenovirus titration.

    You may also use DNase I from bovine pancreas (see Section III). The DNase should be endotoxin-free and chromatographically purified. Add 1000 Kunitz units per 100 ml of solution. Gently mix the solution by inversion, and incubate at 37°C for 30 min.

16. Meanwhile, prepare 1X Dilution Buffer and 1X Wash Buffer by diluting the provided 5X buffers with sterile, Milli-Q H_2O.

Standard Kit	Mega Kit
20 ml 5X Dilution Buffer	100 ml 5X Dilution Buffer
80 ml H_2O	400 ml H_2O
100 ml 1X Dilution Buffer	500 ml 1X Dilution Buffer
12 ml 5X Wash Buffer	60 ml 5X Wash Buffer
48 ml H_2O	240 ml H_2O
60 ml 1X Wash Buffer	300 ml 1X Wash Buffer

17. Mix the Benzonase® Nuclease-treated filtrate with an equal volume of 1X Dilution Buffer.

    Note: The solution may turn faintly purple if phenol red is present. But if you used Tris-HCl to resuspend Pellet 1, you may not observe a color change.

18. Proceed to Part C.

C. Purify Adenovirus

1. Collect the following components and place them within easy reach:
   - Adeno-X Syringe-Filter Assembly (standard) and Tubing Assemblies (Mega)
   - 1X Wash Buffer (Prepared by diluting 12 ml of 5X Wash Buffer with 48 ml of sterile Milli-Q H_2O)
   - 1X Elution Buffer (provided)
   - Sterile PBS
   - 1X Formulation Buffer (provided)
   - 5 ml Luer-Lok™ Tip syringe (provided)
   - Sterile 15 ml or 50 ml centrifuge tube and rack
   - Container for holding biohazardous liquid waste

V. Adenovirus Purification Protocol *continued*

2. Prime the Filter Assembly.

   Note: Air bubbles trapped inside the Filter Purification Cartridge may prevent adenovirus from binding to those areas on the filter.

   - **To prime the standard assembly**
     a. Place the inlet tube into a 50 ml conical tube containing 25 ml of sterile PBS.
     b. Pull the PBS into the syringe (see Figure 2A).
     c. Push the sterile PBS out through the filter at 20 ml/min (drops should be countable)
   - **To prime the Mega assembly**
     a. Connect the assembled purification system to your house vacuum line as shown in Figure 2B.

        Note: Mega Kits include an extra piece of tubing (Tubing Assembly B) with a two-way adaptor for connecting the Purification Filter to your vacuum hose. Be sure that your vacuum source is properly protected with a trap (e.g., a vacuum flask) to collect the filtrate.

     b. Place the Tubing Assembly A (connected to the other end of the filter) into a flask of sterile PBS.
     c. Slowly turn on the vacuum and draw 10–20 ml of sterile PBS through the filter and tubing assembly.
     d. Clamp the tubing, and then turn off the vacuum

4. Place the inlet Tubing in the virus-containing solution (from Step V.B.17) as shown in Figure 2B.
5. Load the virus onto the purificaton filter. Pass the virus-containing solution through the filter at ~20 ml/min (Drops should be countable).
   - **Standard purifications:** Pull the solution through the tubing and the one-way valve into the attached 60 ml syringe (see Figure 2A). When the syringe is full, push the solution through the filter into a biohazard waste container. Repeat these steps until the entire volume has been filtered. Avoid drawing air into the system.
   - **Mega purifications:** Slowly turn on the vacuum. Control the flow rate with the Pinch Clamp (see Figure 2B), or by directly adjusting the vacuum. When you finish filtering the solution, clamp the line firmly and disconnect the vacuum hose.
6. Transfer the inlet Tubing to a holding vessel containing the 1X Wash Buffer.
7. Pass the entire volume of Wash Buffer through the filter at ~20 ml/min.
8. Remove the filter from the Tubing Assembly.

   Note: If you are performing a Mega Purification, remove Tubing Assembly B only (i.e., the tubing that connects the filter to the house vacuum); Tubing Assembly A must remain attached for the elution step that follows.

V. Adenovirus Purification Protocol *continued*

9. Using the clean 5 ml Luer-Lok™ tip syringe, elute the adenovirus as follows:
   - To elute adenovirus from **Standard Filters**, fill a clean 5 ml Luer-Lok™ tip syringe with 3 ml of 1X Elution Buffer; connect the syringe to the female port on the filter cartridge; then push 1 ml of Elution Buffer through the filter into a sterile 15 ml conical centrifuge tube. Incubate the filter at room temperature for 5 min, then push the remaining elution buffer through to collect the remaining adenovirus. Use air to push any remaining virus from the filter.
   - To elute adenovirus from **Mega Filters**, attach a clean, dry 20 ml syringe to the female port on the filter cartridge; then place the tubing assembly, connected to the male port, into a centrifuge tube containing 15–20 ml of 1X Elution Buffer. Using the syringe, pull the buffer from the centrifuge tube through the filter into the syringe. Finally, detach the syringe and save its contents (your purified viral concentrate) in a sterile 50 ml centrifuge tube.

   Notes
   - Luer-Lok™ syringes can properly connect to only one port on the filter cartridge.
   - The Adeno-X Filter has no directionality—i.e., in theory, it does not matter which side you elute from. But we find that this elution protocol is optimal because it helps keep air out of the filter. Air bubbles trapped against the filter may interfere with the elution.
   - The eluate may be faintly pink because of residual phenol red.
10. [Optional] To concentrate the adenovirus even further, or to exchange buffers, transfer the eluate to a Millipore Centrifugal Filter Device.
    a. The Centrifugal Filter Device should have a nominal molecular weight limit (NMWL) of 50,000 and a high-recovery Ultracel-YM Membrane. (See Section III for more information)
    b. Follow the instructions provided with the device. Do not allow the volume to be reduced below 0.5 ml, as this may cause the virus to aggregate. When the volume has been reduced to 1 ml, add 2–3 ml of 1X Formulation Buffer and centrifuge again.
11. Determine adenoviral titer.

    Note: We recommend using the Adeno-X™ Rapid Titer Kit (Cat. No. 631028).
12. Aliquot and store the adenovirus at –70°C. But first see the note below.

    Note: Although adenovirus can be stored in 1X Elution Buffer, we recommend you use 1X Formulation Buffer, as it contains a cryoprotectant for stability during freeze-thaw, salt to prevent aggregation and maintain isotonicity, and Tris buffer to maintain pH over a range of temperatures (Hoganson, *et al.*, 2002). The composition of 1X Formulation Buffer is the same as that used for the Ad 5 WT Reference Material (Hutchins, B., 2002). To exchange 1X Elution Buffer for 1X Formulation Buffer, follow Steps 10.a–10.b.
13. The purified adenovirus can now be used for *in vivo* or *in vitro* experiments.

VI. References

Adeno-X Virus Purification Kits (July 2002) *Clontechniques* **XVIII**(3):10–11.

Adeno-X Rapid Titer Kit (April 2002) *Clontechniques* **XVII** (2):16–17.

Graham, F. L. & Prevec, L. (1991) Manipulation of adenovirus vectors. In *Methods in Molecular Biology, Vol. 7: Gene Transfer and Expression Protocols.* Ed. Murray, E. J. (Human Press Inc., Clifton, NJ), pp. 109–128.

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Hutchins, B. (2002) Development of a reference material for characterizing adenovirus vectors. *Bioprocessing J.* **1**(1):25–28.

VII. Related Products

For a complete listing of all Clontech products,
please visit **www.clontech.com**

Products	Cat. No.
• Adeno-X™ Expression System 1	631513
• Adeno-X™ Tet-Off Expression System 1	631022
• Adeno-X™ Tet-On Expression System 1	631050
• Adeno-X™ Expression System 2	631524
• Adeno-X™ Tet-On Expression System 2	631057
• Adeno-X™ Tet-Off Expression System 2	631058
• Adeno-X™ Promoterless Expression System 2	631525
• Adeno-X™ Rapid Titer Kit	631028
• Adeno-X™ PCR Screening Primer Set	631030
• Adeno-X™ -DsRed2 Adenovirus	632417
• Adeno-X™-LacZ Adenovirus	631029
• Adeno-X™ -Null Adenovirus	631517
• Adeno-X™ Accessory Kit	631027
• RNAi-Ready pSIREN-Shuttle Vector	631527
• Knockout Adenoviral RNAi System 1	631528
• Knockout Adenoviral RNAi System 2	631529

Notes

Notice to Purchaser

This product is intended to be used for research purposes only. It is not to be used for drug or diagnostic purposes nor is it intended for human use. Clontech Laboratories, Inc. products may not be resold, modified for resale, or used to manufacture commercial products without written approval of Clontech Laboratories, Inc.

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