AdEasy Virus Purification Kit 500

Instruction Manual

Catalog #240245 (1 prep × 500 ml)

Revision E.0

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240245-12

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AdEasy Virus Purification Kit 500

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MATERIALS PROVIDED

Materials Provided	Quantity
Sartobind® Syringe Filter Unit	1
Sartopore® Clarifying Unit	1
Centrifuge Concentrator	2
10 ml Syringe	1
10× Loading Buffer	60 ml
10× Washing Buffer	30 ml
Elution Buffer	20 ml
Tubing Set (Masterflex® L/S 16–size tube)	2

STORAGE CONDITIONS

Store all components at room temperature

ADDITIONAL MATERIALS REQUIRED

Benzonase® Nuclease, purity >90%, 2.5 KU (EMD Biosciences, Catalog #70746-4)
Centrifuge with rotor accepting 50-ml conical tubes
Peristaltic pump accepting Masterflex® L/S 16-size tube
Retort stand and clamp
Phosphate-buffered saline (PBS), pH 7.4[§]
DMEM complete growth medium[§]
Ethanol / dry ice bath or –80°C freezer
Water bath at 25°C
Storage Buffer (optional)[§]

[§] See *Preparation of Media and Reagents*

Revision E.0

INTRODUCTION

The AdEasy Virus Purification Kit allows the purification and concentration of Adenovirus (from Ad5 strains) with Sartobind® syringe filters containing an ion exchange membrane adsorber that selectively binds adenoviral particles. Once bound, viral particles can be further purified by washing away nonspecifically-bound proteins before elution. Concentrated and purified viral particles can be obtained in 2–3 hours, in contrast to traditional CsCl gradient centrifugation which typically takes 12–48 hours (see Table 1). Ready-to-use Sartopore® filter units, Sartobind syringe filters, centrifuge concentrators, and buffers make the AdEasy virus purification procedure easy and efficient. For a typical adenoviral vector, 500 ml of cell culture (25 × 15-cm^2 plates) purified using this method should yield approximately 1–3 × 10^{13} viral particles.

TABLE 1

Comparison of Purification Protocols (500-ml Cultures)

Purification Method	Process Time	Recovery[a]	Eluate Volume[b]	Viral Particle Yield
AdEasy Virus Purification Kit	2–3 hours	80%	1 ml	1–3 × 10^{13}
CsCl gradient	12–48 hours	60–70%	1–2 ml	1 × 10^{13}

[a] before buffer exchange

[b] after buffer exchange

Overview of the AdEasy Purification Kit Protocol

This protocol is used to concentrate and purify adenovirus type 5 strains. The protocol is shown in Figure 1 and summarized below. A detailed protocol follows in the *Purification of Adenovirus Protocol* section.

Note *This kit contains sufficient materials to concentrate and purify virus from 500 ml culture medium, and the detailed protocols are written for a 500-ml preparation. Please adjust reagent volumes accordingly for smaller samples.*

Virus Culture

Infect AD-293 or HEK293 cells (typically, 500 ml of cell culture grown in 25 × 15-cm^2 plates) with Adenovirus stock and grow the cells for 3–5 days until most cells show full cytopathic effects. The cells will round up and detach.

Sample Preparation

Harvest the cells by centrifugation, retaining both the pellet and supernatant. Resuspend the pellet in 25 ml of supernatant and lyse the cells by 3 freeze/thaw cycles. Centrifuge to remove cellular debris, and then recombine with the reserved supernatant. Digest unwanted nucleic acids with Benzonase® nuclease. Filter the nuclease-treated supernatant using the Sartopore filter unit. Add Loading Buffer to a final 1× concentration.

Sartobind® Filter Unit Preparation

Equilibrate the membrane with PBS and remove air bubbles from the Sartobind unit, before loading virus. (Failure to remove all the air bubbles will reduce the binding of virus to the filter membrane).

Sample Loading

Pass the prepared supernatant slowly drop-by-drop through the Sartobind unit. Using the correct flow rate is critical. For maximum binding of virus, load at no more than 10 ml/minute.

Filter Washing

Wash away residual culture medium, contaminating proteins and nucleic acids. A higher flow rate of 10–20 ml/minute may be used for washing.

Elution

Elute purified viral particles by passing Elution Buffer through the Sartobind unit with a syringe. Incubation of the Sartobind unit with Elution Buffer, and using the correct flow rate during elution are critical. For maximum recovery of viral particles, elute at no more than 1 ml/minute.

Concentration/Buffer Exchange

Virus concentration may be increased using the Centrifuge Concentrators supplied with this kit. If desired, the Centrifuge Concentrators may also be used to exchange Elution Buffer for appropriate physiological or storage buffer.

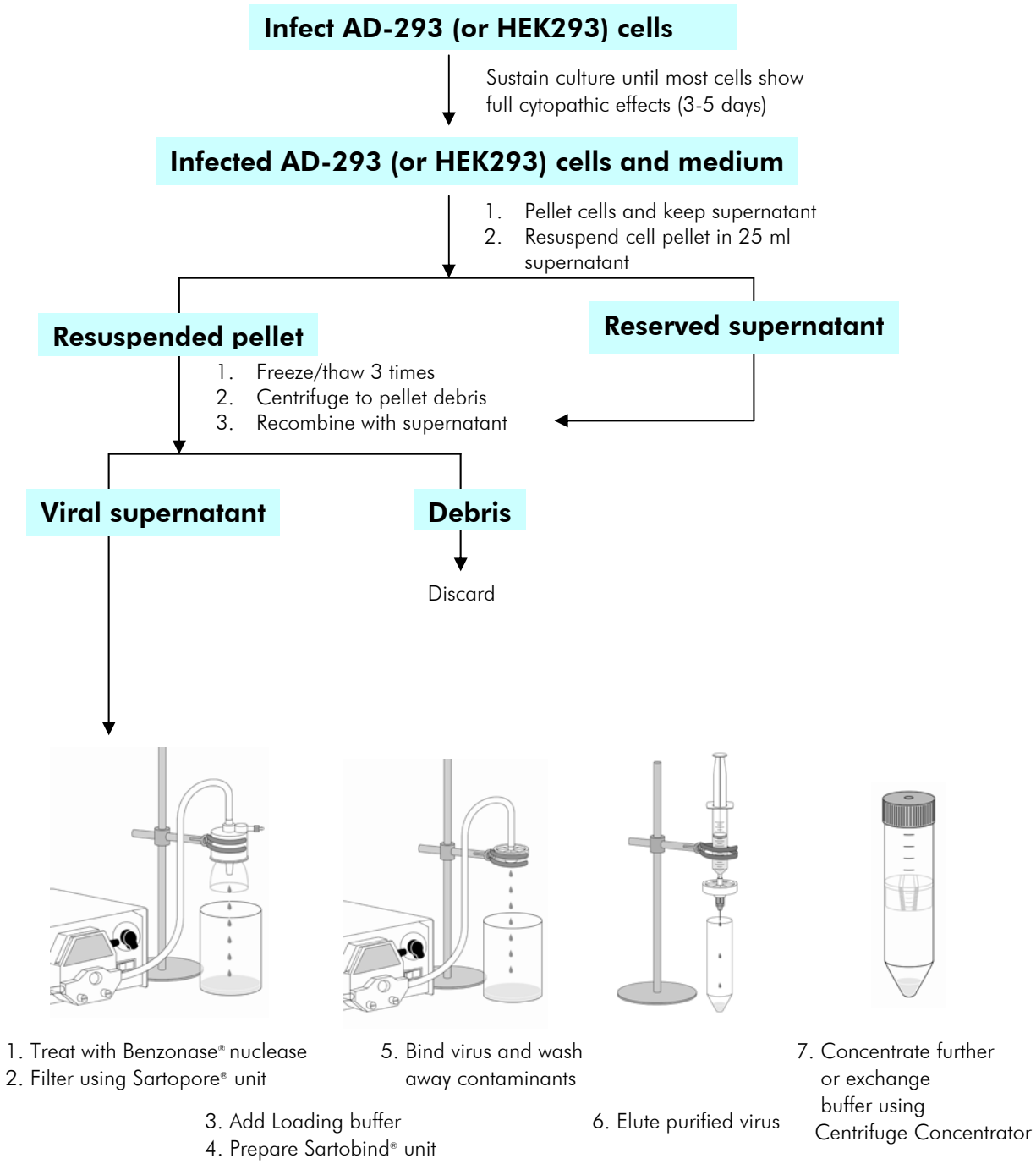

Figure 1 Overview of purification protocol.

PURIFICATION OF ADENOVIRUS PROTOCOL

Virus Culture

Seed up to 25 × 15-cm^2 plates with AD-293 or HEK293 cells in 20 ml DMEM with 10% FBS (see *Preparation of Media and Reagents*) per plate and grow at 37°C with 5% CO_2. When the cell monolayer reaches a confluency of 60–80%, infect cells with an Ad5 type adenovirus stock at an m.o.i. of at least 20. Culture the infected cells at 37°C with 5% CO_2 for 3–5 days until most cells show cytopathic effects. The cells should round up and detach, but it may be necessary to detach adhering cells using a cell scraper.

Note *The 10× Loading Buffer included in the AdEasy kit is specially formulated to be used with the specified culture conditions, please follow them carefully.*

Sample Preparation

1. Pool infected cells and medium. Pellet cells by centrifugation at 3,500 × *g* for 15 minutes.

2. Decant supernatant to a sterile container and store at 4°C.

3. Resuspend the cell pellet in 25 ml of the reserved supernatant.

4. Freeze/thaw cell suspension 3 times to disrupt cells using alternately an ethanol/dry ice bath or –80°C freezer and a water bath set at 25°C.

   Note *Do not allow the temperature to rise above 25°C at any time.*

5. Pellet debris by centrifugation at 3,500 × *g* for 15 minutes at room temperature. Decant supernatant and add to the reserved supernatant from step 2.

6. Add 12.5 U of Benzonase nuclease for each milliliter of supernatant, for a final concentration of 12.5 U/ml. Mix sample and incubate at 37°C for 30 minutes.

7. Dilute 10× Washing Buffer to 1× working concentration by adding 30 ml to 270 ml deionized water and mixing well. Set aside until later.

Sartopore® Filter Clarification of Viral Supernatant

1. Assemble equipment as shown in Figure 2. Loosen the hydrophobic vent plug in the Sartopore filter casing. Pump the Benzonase nuclease-treated supernatant through the filter at 10–20 ml/minute. Any air bubbles trapped in the capsule housing can escape through the hydrophobic vent. Close the vent plug and pump the liquid out of the filter capsule.

2. Add the appropriate volume of 10× Loading Buffer to the Benzonase nuclease-treated supernatant slowly while agitating to achieve a final 1× buffer concentration. (e.g., add 55 ml 10× Loading Buffer to 500 ml supernatant).

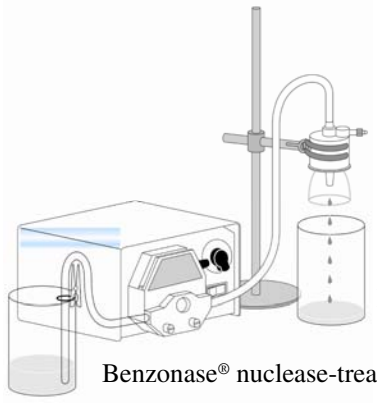

Figure 2 Sartopore® filter equipment assembly.

Sartobind® Filter Unit Purification of Adenovirus

Prepare Sartobind® Filter Unit

Note *Air trapped in the Sartobind unit will reduce viral titer. All of the air must be removed from the Sartobind unit so that virus particles can bind to the membrane.*

1. Using a fresh tube set, assemble the equipment as illustrated in Figure 3A.
2. Pour 300 ml PBS into the beaker.
3. Pump PBS through the Sartobind unit to ensure it is fully wetted.
4. Adjust the pump setting until a flow rate of 10 ml/minute is achieved.
5. Pump through 30–50 ml PBS then stop the pump using the switch; do not adjust the pump speed.

   Note *Loading too quickly will reduce the capture of virus particles and may result in decreased viral titer.*

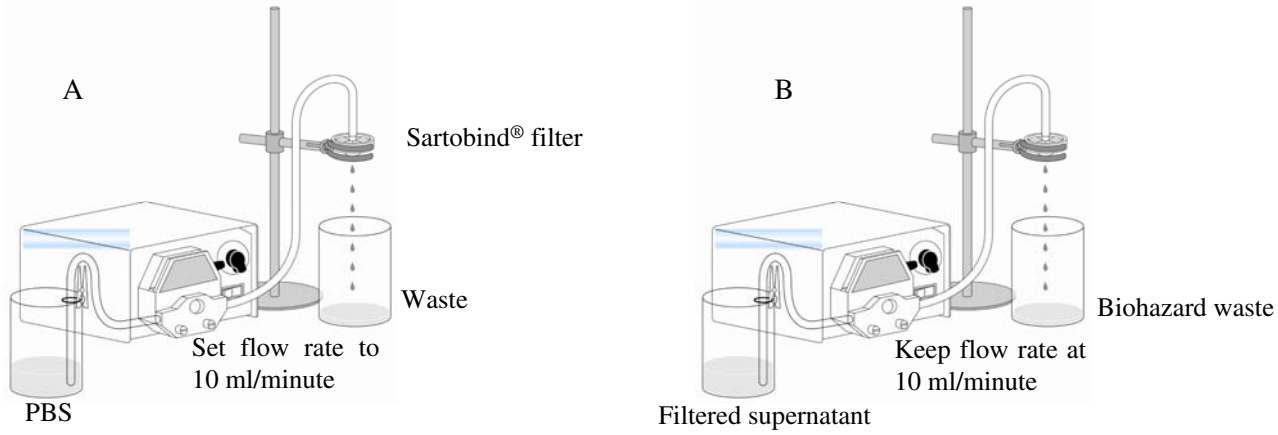

Figure 3 Sartobind® filter equipment assembly.

Sample Loading and Washing

1. Remove the feed tube from the beaker containing PBS and place into the container of prepared viral supernatant. Pump through the Sartobind unit at the set speed of 10 ml/minute. Collect flow through and treat as biohazard waste (see Figure 3B).

2. When the supernatant container is almost empty, pour 300 ml 1× Washing Buffer into the sample container.

3. Pump the Washing Buffer through the filter at 10–20 ml/minute.

Elution

1. Fill a fresh 10-ml syringe with 10 ml Elution Buffer and set aside.

2. Detach the Sartobind unit from the tubing assembly and attach the filled 10-ml syringe to the inlet.

3. Hold the syringe vertically. Very slowly (drop-by-drop) pass 1 ml Elution Buffer through the Sartobind unit and collect in a sterile 15-ml tube (see Figure 4).

   Note *Press syringe plunger very gently. Eluting too quickly will reduce the recovery of purified virus. The optimal flow rate for elution is 1 ml/minute; you will achieve this if you can count the individual drops.*

4. Leave the syringe (with the remaining 9 ml Elution Buffer in it), attached to the Sartobind unit and incubate for 5–10 minutes at room temperature.

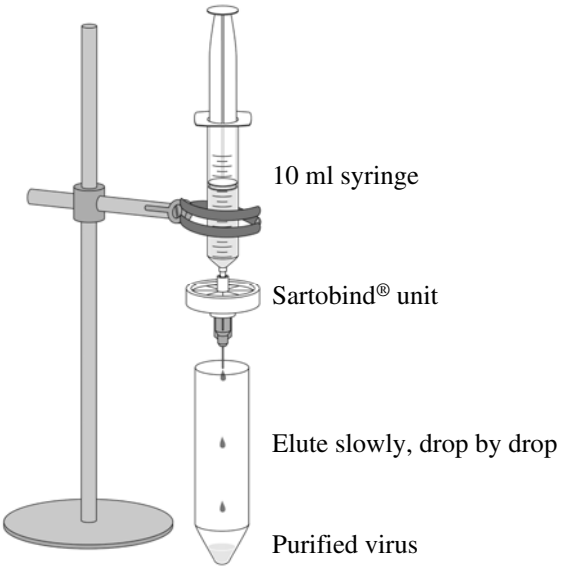

Figure 4 Elution set up.

5. Pass the remaining Elution Buffer through the Sartobind unit very slowly as before. When the bound virus has been fully eluted, the membrane will appear white. If the membrane remains pink, repeat steps 1–5.

6. Finally using the syringe, push air slowly through the unit to recover as much of the eluate as possible.

Viral Eluate Concentration and Buffer Exchange (Optional)

Note *Further concentrate the viral eluate to increase infectivity. It is recommended that virus is exchanged into physiological buffer before use in tissue culture or cell based assays, or into generic storage buffer for long-term storage at –80°C. Storage buffers containing glycerol may take considerably longer to concentrate than the original viral eluate solution. Centrifuge at 4°C to prevent overheating during longer centrifugation times.*

1. Transfer eluate to a Centrifuge Concentrator (see Figure 5) and counterbalance the rotor with a second concentrator filled with an equivalent volume of PBS or water. In fixed angle rotors, the printed graduations should face away from the center of the rotor.

2. Centrifuge for 15 minutes at room temperature at up to 3,000 × *g* in a swing-out rotor, or 6,000 × *g* in a 25° fixed-angle rotor, with cavities accepting 50-ml conical-bottom tubes.

3. Check the volume of viral concentrate remaining in the upper chamber and if necessary centrifuge again.

   Note *Do not reduce the volume to less than 1 ml in order to avoid virus aggregation and loss of infectivity.*

4. If exchanging buffers, discard filtrate when sample volume reaches 1 ml, and then add 4 ml of storage/physiological buffer (see *Preparation of Media and Reagents*) to the concentrate to bring the volume up to 5 ml. This will bring the virus to normal physiological conditions. Centrifuge again as before to concentrate. If necessary, repeat buffer exchange a second time.

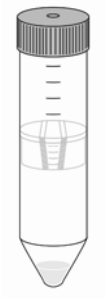

Figure 5 Centrifuge Concentrator.

5. Recover the concentrated virus by pipet. Resuspend concentrated virus by gently pipetting up and down a few times before recovery.

6. Aliquot and store virus at –80°C. Once thawed, keep at 4°C and do not re-freeze. Virus should remain viable for up to 2 years at –80°C when purified by this procedure.

7. Determine viral titer. The AdEasy Viral Titer Kit (Catalog #972500) provides a rapid, efficient method for titer determination.

Typical Performance

For a typical vector, 500 ml of infected cell culture (25 × 15-cm^2 culture plates) purified using this method should yield a range of up to 1–3 × 10^{13} viral particles. Values may be different depending on individual conditions.

TROUBLESHOOTING

Observations	Suggestions
Air in the feed tube	Do not expel through the Sartobind units. Remove the Sartobind unit temporarily from the syringe and expel the air. Re-fill the syringe and tube set with liquid then re-fit Sartobind unit.
	Ensure the tube holder is firmly clipped onto the side of the flask and that the end of the feed tube is sitting in the liquid.
Low virus recovery	Optimize virus production.
	Harvest virus cultures when cytopathic effects are obvious in the majority of cells. Cultures that are allowed to grow too long may result in decreased titers.
	Avoid trapping air in the Sartobind unit.
	Load at no more than 10 ml/minute.
	Elute at no more than 1 ml/minute.
	Confirm that correct buffers were used.
	After elution, push air through the Sartobind unit to recover all the buffer.
Sartopore unit clogs during filtration	Loosen hydrophobic vent plug to allow air bubbles to escape.
	Centrifuge sample at 3,500 × *g* for 15 minutes to pellet cellular debris prior to clarification through Sartopore filter.
Sartobind unit clogs during filtration	Centrifuge sample at 3,500 × *g* for 15 minutes to pellet cellular debris prior to clarification through Sartopore filter.

PREPARATION OF MEDIA AND REAGENTS

PBS	Storage Buffer
137 mM NaCl 2.6 mM KCl 10 mM Na_2HPO_4 1.8 mM KH_2PO_4 Adjust the pH to 7.4 with HCl	20 mM Tris/HCl 25 mM NaCl 2.5% Glycerol (w/v) Adjust the pH to 8.0 at 22°C with HCl
Complete DMEM Growth Medium	
DMEM (containing 4.5 g/L glucose, 110 mg/L sodium pyruvate, and 2 mM L-glutamine) supplemented with 10% (v/v) heat-inactivated fetal bovine serum	

ENDNOTES

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Benzonase® is a registered trademark of Merck KGaA, Darmstadt, Germany.
Masterflex® is a registered trademark of Cole-Parmer Instrument Company.

Sartobind® filtration units are manufactured by Sartorius for Stratagene Products Division. (*Sartobind Inside*).

MSDS INFORMATION

Material Safety Data Sheets (MSDSs) are provided online at *http://www.genomics.agilent.com*. MSDS documents are not included with product shipments.