AdEasy Virus Purification Kits

2 × 100 and 5 × 100

Instruction Manual

Catalog #240243 (2 preps × 100 ml), #240244 (5 preps × 100 ml)

Revision G0

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240243-12

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AdEasy Virus Purification Kits

Contents

AdEasy Virus Purification Kits

MATERIALS PROVIDED

Materials Provided	Catalog #240243 (2×100)	Catalog #240244 (5×100)
Sartobind® Syringe Filter Unit	2	5
0.45 μm Filter	4	10
Centrifuge Concentrator	4	10
10 ml Syringe	4	10
50 ml Syringe	2	5
Elution Tip	2	5
10× Loading Buffer	25 ml	60 ml
Washing Buffer	120 ml (1× concentration)	60 ml (10× concentration)
Elution Buffer	20 ml	30 ml
Tubing Set (Masterflex® L/S 16–size tube)	2	5

Note *The AdEasy kit 2 × 100 contains sufficient materials to concentrate and purify virus from 2 × 20–100 ml cultures or 1 × 100–200 ml culture. The AdEasy kit 5 × 100 contains sufficient materials to concentrate and purify virus from 5 × 100 ml cultures.*

STORAGE CONDITIONS

Store all components at room temperature.

ADDITIONAL MATERIALS REQUIRED

Benzonase® Nuclease, purity >90%, 2.5 KU (EMD Millipore, Catalog #70746-4)
Centrifuge with rotor accepting 15-ml and 50-ml conical tubes
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*

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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 0.45 µm filters, Sartobind filter units, centrifuge concentrators, and buffers make the AdEasy virus purification procedure easy and efficient. For a typical adenoviral vector, 60 ml of cell culture (3 × 15-cm plates) purified using this method should yield approximately $1–3 \times 10^{12}$ viral particles with an endotoxin level of <0.025 EU/ml.

TABLE 1

Comparison of Purification Protocols

Purification Method	Process Time	Recovery[a]	Eluate Volume[b]	Viral Particle Yield
AdEasy Virus Purification Kit (60 ml)	1–2 hours	65%	1 ml	$1–3 \times 10^{12}$
AdEasy Virus Purification Kit (200 ml)	2–3 hours	80%	1 ml	1×10^{13}
CsCl gradient (500 ml culture)	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 *The AdEasy kit 2 × 100 contains sufficient materials to concentrate and purify virus from 2 × 20–100 ml cultures or 1 × 100–200 ml culture. The AdEasy kit 5 × 100 contains sufficient materials to concentrate and purify virus from 5 × 100 ml cultures. Please follow the proper protocol for your sample size.*

Virus Culture

Infect AD-293 or HEK293 cells in 15-cm plates with Adenovirus stock and grow the cells 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 10 ml of supernatant and lyse the cells by three 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 0.45 µm 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 filter unit before loading virus. Use a single unit for up to 100 ml virus culture, or use two units in tandem for up to 200 ml virus culture. (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 with the wash buffer. 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 using the special Elution Tip. 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 the kit. If desired, the Centrifuge Concentrators may also be used to exchange Elution Buffer for appropriate physiological or storage buffer.

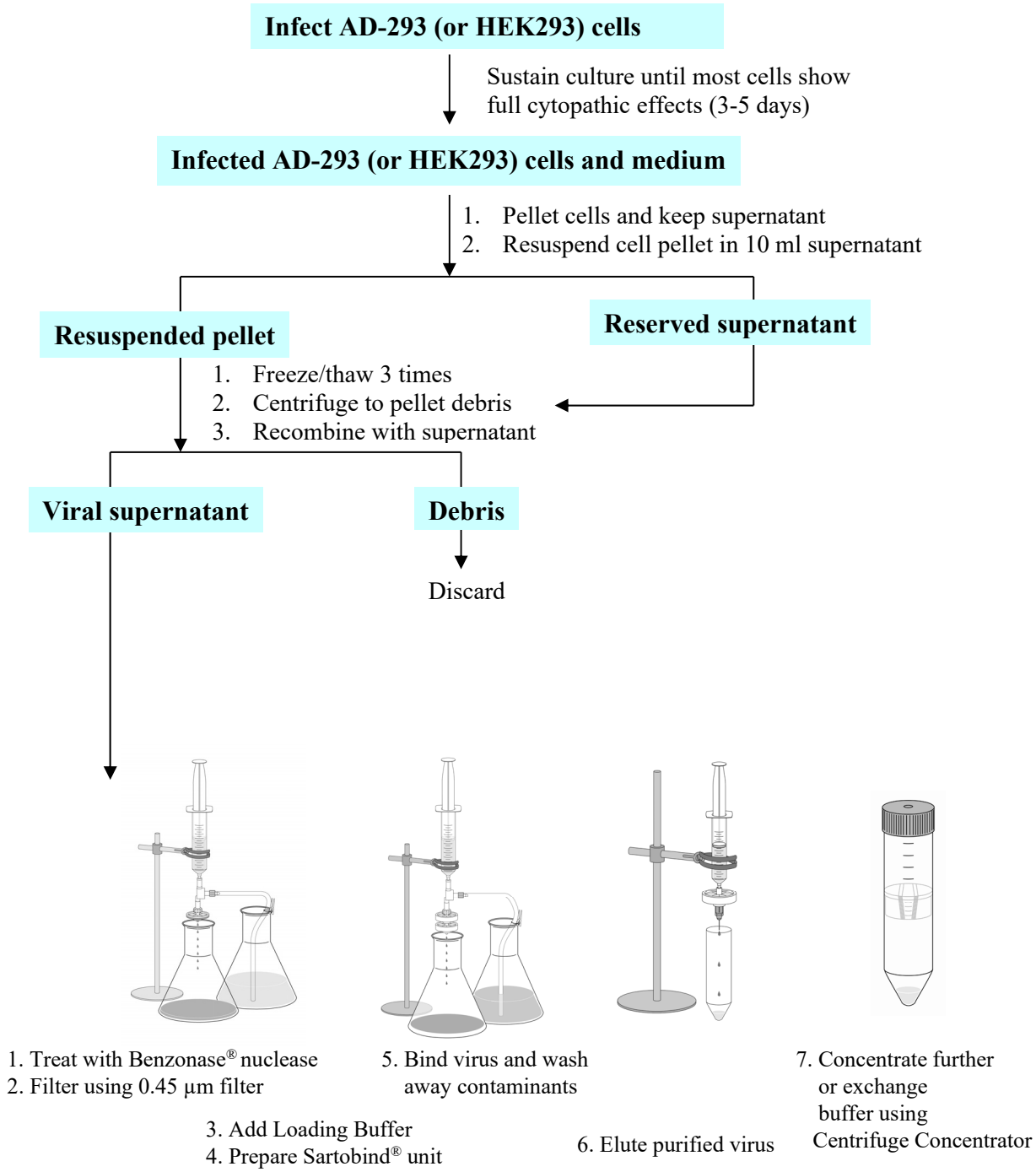

Figure 1 Overview of purification protocol.

PURIFICATION OF ADENOVIRUS PROTOCOL

Virus Culture

Seed the desired number of 15-cm plates with AD-293 or HEK293 cells in DMEM with 10% FBS (see *Preparation of Media and Reagents*) 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 10–20. 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.

Notes *For maximum recovery and ease-of-use, a culture volume of 60 ml is recommended per Sartobind unit (60 ml for a single unit and 120 ml for two units in tandem). Culture volumes may be increased to up to 100 ml per unit, but the recovery per ml culture may be reduced.*

The 10× Loading Buffer included in the AdEasy kit is specially formulated to be used with the specified culture conditions.

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 10 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. 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.

0.45 μm Filter Clarification of Viral Supernatant

1. Attach the tube set to the 50-ml syringe and clamp this to a retort stand as shown in Figure 2A.

2. Place the feed tube into the supernatant and draw some liquid up into the syringe (a). Push this liquid, and the air in the syringe, out through the one-way valve back into the container (b). Repeat until all the air is removed from the syringe.

3. Fill the syringe with Benzonase nuclease-treated supernatant and attach a 0.45 μm filter to the syringe assembly. (See Figure 2B.)

4. Filter the whole volume into a fresh container. Leave 1–2 ml liquid in the syringe each cycle (to prevent air entering the filter).

   Note *Once wetted, do not push air through the filter during filtration as this may block the filter. If air is drawn into the feed tube, see* Troubleshooting*. If the 0.45 μm filter unit becomes blocked, replace it and continue with the filtration.*

5. Add 1/9 volume of 10× Loading Buffer to the filtered supernatant slowly while agitating to achieve a final 1× buffer concentration (e.g., add 7 ml 10× Loading Buffer to 60 ml supernatant).

6. Remove and discard the 0.45 μm filter.

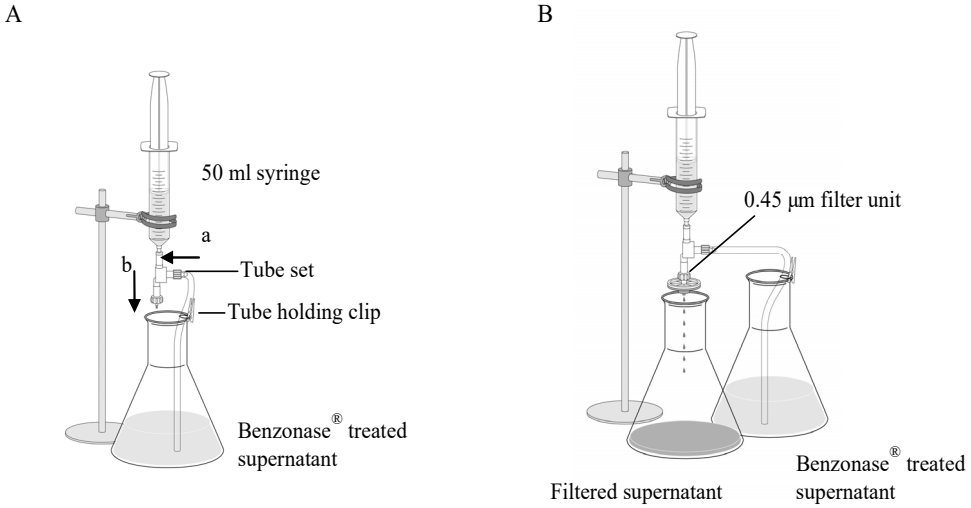

Figure 2 0.45 μm filter equipment assembly.

Sartobind® Filter Purification of Adenovirus

Prepare Sartobind® Filter Unit

Notes *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.*

Use a single Sartobind unit for 20–100 ml culture volumes, or use two Sartobind units in tandem for 100–200 ml culture volumes (see step 7, below, for tandem unit assembly).

1. Fill a fresh 10-ml syringe with >10 ml PBS.
2. Remove and keep the protective cap from a single Sartobind unit. Attach the Sartobind unit to the filled 10-ml syringe as shown in Figure 3A.
3. Apply gentle pressure to the plunger and rinse through ~8 ml of PBS.
4. Pump the syringe plunger gently up and down a few times, to remove air from the Sartobind housing.
5. Once all the air has been removed from the Sartobind housing, continue applying moderate pressure and flush through all except the last 2 ml of PBS.
6. Leaving 2 ml of PBS in the syringe, replace the cap on the outlet of the filled Sartobind unit and remove it from the syringe. Keep upright as much as possible and set it aside.

Note *The outlet of the Sartobind unit needs to be capped before it is removed from the syringe to prevent introducing air into the unit.*

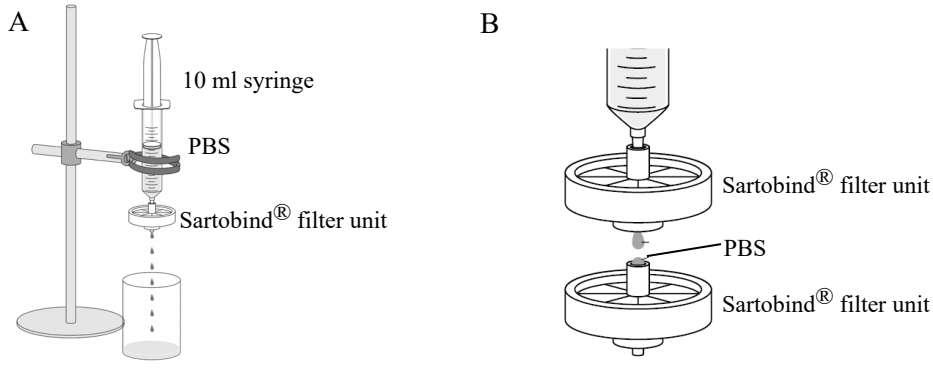

Figure 3 Sartobind® filter equipment assembly

7. If you are processing 100–200 ml of Adenoviral culture, repeat the process using a second Sartobind unit, but do not remove this unit from the syringe. The units need to be connected "wet to wet" to avoid trapping air between the devices. Add 100 µl PBS to the inlet of the unit previously prepared and attach it to the outlet of the unit attached to the syringe. (See Figure 3B).

Sample Loading

Note *It is important to hold the assembly vertical and steady throughout sample loading. This is easier if the filled syringe assembly is clamped to a retort stand before loading.*

1. Take the 50-ml syringe and tube set previously used for filtration. Place the end of the feed tube into the prepared sample solution (see Figure 4A). Remove the air from the syringe and valve. (See step 2 under *0.45 µm Filter Clarification.*) Fill the syringe with filtered supernatant.

2. Fit the wetted Sartobind unit(s) to the outlet, taking care not to trap any air bubbles (wet-to-wet connection). (See Figure 4B.)

3. Pass prepared sample solution slowly through the Sartobind unit(s). The optimal flow rate for loading is 10 ml/minute; you will achieve this if you can count the individual drops. Leave 1–2 ml liquid in the syringe at the end of each cycle to prevent air entering the Sartobind unit.

   Note *Press syringe plunger gently. Loading too quickly will reduce the capture of virus particles.*

4. Continue until a minimum of sample is left in the sample container but the feed tube remains full, then continue to the washing step.

   Note *Do not draw air into the feed tube. If this happens, see* Troubleshooting *section.*

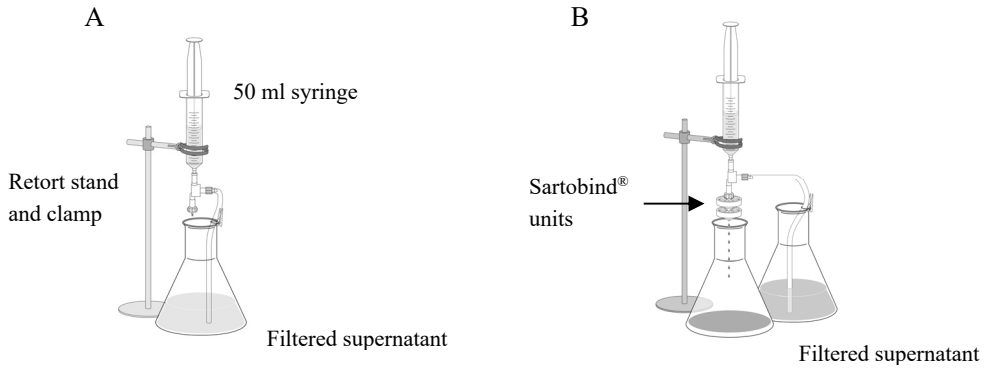

Figure 4 Sample loading equipment

Sample Washing

Notes *If using AdEasy 5 × 100 kit, dilute 10× Washing Buffer to 1× working concentration by adding 10 ml to 90 ml deionized water (for each 100-ml culture preparation) and mixing well.*

To ensure an efficient changeover from loading to washing, draw up sufficient Washing Buffer to just fill the feed tube, then push out through the Sartobind unit to flush the remaining sample solution through before continuing with the main wash.

1. Pour Washing Buffer into the almost empty sample container. Use the same volume as your original culture volume. However, Washing Buffer volumes larger than 60 ml are not necessary.

2. Pass the Washing Buffer through the Sartobind unit(s). The flow rate for washing may be higher than for loading (10–20 ml/minute).

   Note *Do not push air through the Sartobind unit(s) during washing.*

3. Leave 1–2 ml liquid in the syringe at the end to prevent air entering the Sartobind unit(s) and continue to the elution step.

Elution

Note *If 2 Sartobind units were used in tandem to purify up to 200 ml of virus culture, separate and elute from each unit individually. The majority of the virus will be bound to the upper unit.*

1. Take a 10 ml syringe and fill with 5 ml Elution Buffer. Remove any air bubbles.

2. Detach a Sartobind unit from the 50 ml syringe and tube set and attach to the filled 10 ml syringe.

3. Fit the red Elution Tip to the outlet of the Sartobind unit. This tip makes it easier to obtain the optimum flow rate for elution.

   Note *Improper use of the Elution Tip may result in shearing of the viral particles. Be sure to elute at 1 ml/minute.*

4. 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 5.)

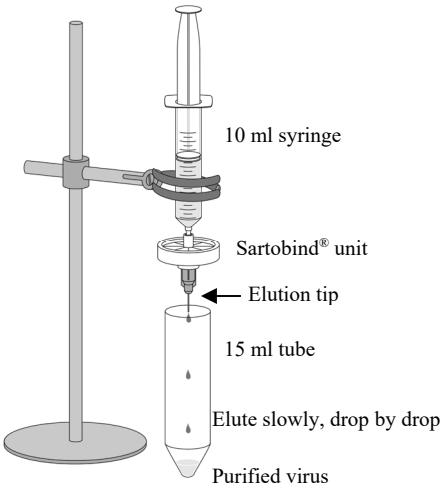

Figure 5 Elution set up.

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.*

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

6. Pass the remaining Elution Buffer through the Sartobind unit very slowly as before.

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

8. Elute the second Sartobind unit (if used), into the same 15 ml tube, repeating steps 1 to 7.

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 6) 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 to concentrate. If necessary, repeat buffer exchange a second time.

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

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

7. 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.

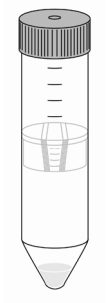

Figure 6 Centrifuge Concentrator.

Typical Performance

For a typical vector, 60 ml of infected cell culture (3 × 15-cm culture plates) purified using this method should yield a range of up to 1–3 × 10^{12} 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.
0.45 μm filter clogs during filtration	Avoid pushing air through the filter once it is wetted.
	Centrifuge sample at 3,500 $\times$ *g* for 15 minutes to pellet cellular debris prior to clarification through filter.
Sartobind unit clogs during filtration	Centrifuge sample at 3,500 $\times$ *g* for 15 minutes to pellet cellular debris prior to clarification through 0.45 μm filter.

PREPARATION OF MEDIA AND REAGENTS

PBS

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

Storage Buffer

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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