



Vivapure[®] AdenoPACK[™] 100

Adenovirus (Ad5) purification and concentration kit for
up to 200 ml cell culture volume (E.g. 1 - 10 x 15 cm plates)

Now with
more capacity for
the same price!

Technical data and operating instructions.
For *in vitro* use only.

Warning: The virus purified using this kit is capable of infecting human or animal cells and could, depending on the gene insert, expose the user to potentially hazardous biological material. Adenoviruses have been designated as Level 2 biological agents. All protocols detailed in these operating instructions must be performed under at least Biosafety Level 2 working condition this kit is NOT intended for human or animal diagnostic or therapeutic applications.

Vivapure AdenoPACK 100 - Introduction

Storage conditions / shelf life



Caution: Benzonase® should be removed from the kit and stored at -20°C immediately.

The remaining AdenoPACK kit contents should be stored at room temperature.

This kit should be used within 12 months of purchase.



Technical assistance

For more information, please contact the Vivascience Support Center.

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Introduction

This protocol describes the purification of Adenovirus (Ad5 strains) with AdenoPACK syringe filters containing an ion exchange membrane adsorber that binds adenoviral particles. Once bound, virus particles can be further purified by washing away non-specifically bound proteins, before elution within 1 - 2 hours.

In contrast, traditional CsCl gradient centrifugation is a time consuming method, typically taking 1 - 2 days. Furthermore, the toxicity of the media places limitations on downstream applications.

Ready to use filter devices, AdenoPACK units, centrifugal Vivaspin concentrators and buffers make the following purification procedure as easy as filtration.

Vivapure AdenoPACK 100	
Cat. Number	VS-AVPQ101
Number of purifications possible with AdenoPACK 100	2 x 20 - 60 ml or 1 x 200 ml
AdenoPACK membrane adsorber unit	2
Elution tip (Red)	2
Minisart plus 0.45 µm SFCA + GF	4
50 ml syringe	2
10 ml syringe	4
Tubing set	2
Loading Buffer (10X)	25 ml
Washing Buffer	120 ml
Elution Buffer	20 ml
Benzonase®* (12.5 U/µl)	200 µl
Vivaspin 20, 100 kDa MWCO	4
Technical data sheet	2

Materials of construction	
AdenoPACK MA housing	Polysulfone
Minisart housing	Cryolite
AdenoPACK membrane	Stabilised RC
Buffer containers	LDPE
Purification buffers	Proprietary

Kit specifications	
Sample size	20 to 200 ml of Adenovirus supernatants
Virus particles (VP) per ml	Typically up to 1×10^{13}
VP/IU	20 - 50
Processing time	Typically 2 hours
Endotoxin level	<0.025 EU/ml

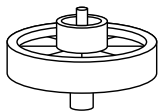
Virus purification tests conducted in cooperation with Progen Biotechnik GmbH, Heidelberg.



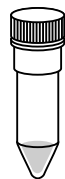
Providing custom made vector development and production service for Adenovirus and Adeno-associated Virus vector systems.

*Benzonase® is a trademark of Merck KGaA, Darmstadt, Germany.

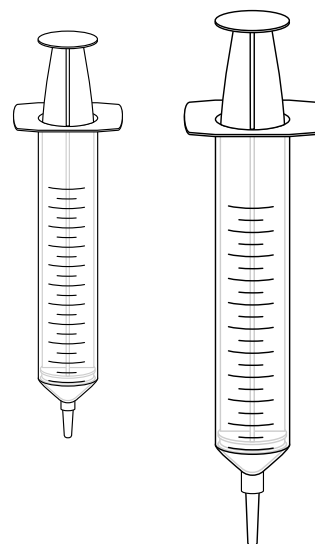
Kit contents



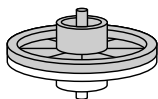
2 x AdenoPACK units
with protective end caps



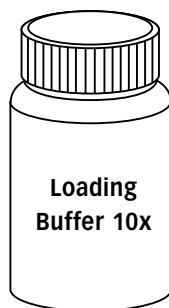
1 x Benzonase® stock (12.5 U/μl)
Store at -20°C



2 x 50 ml syringes &
4 x 10 ml syringes



4 x Minisart plus
in blister packs (Yellow)



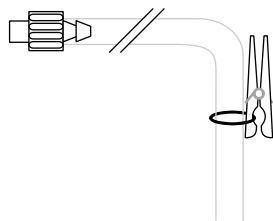
1 x 25 ml Loading Buffer



2 x Elution tips (Red)



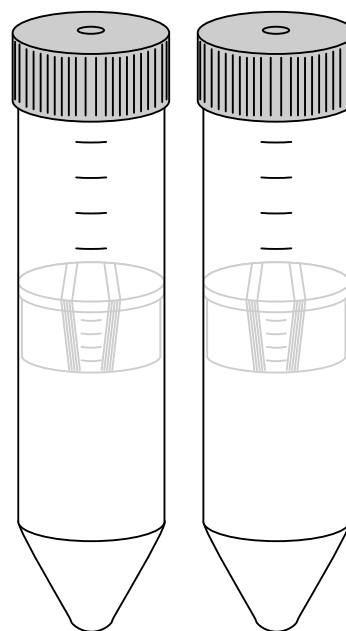
1 x 120 ml Washing Buffer



2 x Tube set with one-way valve



1 x 20 ml Elution Buffer



4 x Vivaspin 20 concentrators with
100 kDa membrane

Additional material required but not supplied

Centrifuge with fixed angle or swing out rotors accepting 15 ml and 50 ml falcon tubes.

100 ml Phosphate buffered Saline pH 7.4 (PBS)

Ethanol / dry ice bath or -80°C freezer

Water bath at 25°C

Retort stand and clamp

Sterile 250 ml plastic container for sample handling

150 ml plastic beaker for rinsing

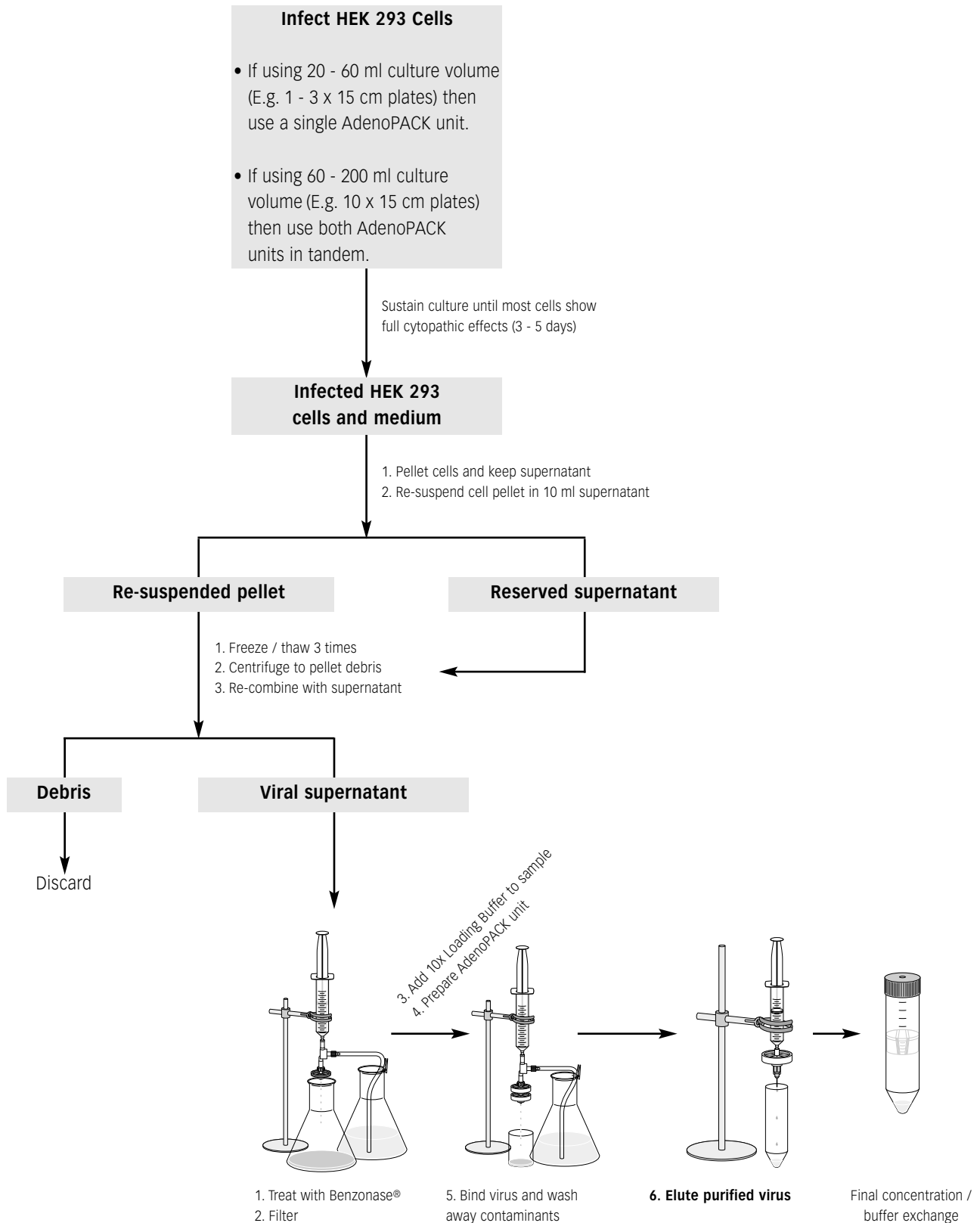
500 ml plastic beaker for collecting loading and washing waste

Sterile 15 ml collection / reaction tube for collection of purified virus

Optional - Storage Buffer: 20 mM Tris/HCl, 25 mM NaCl,

2.5% Glycerol (w/v), pH 8.0 at 22°C

Purification protocol - Overview



Purification protocol - Overview

General protocol

The protocol uses the following steps to concentrate and purify adenovirus type 5 strains.

Note: This kit contains sufficient materials for 2 x 20 - 60 ml preparations or 1 x 60 - 200 ml preparation. The detailed protocols are written as though for a single 200 ml preparation, please adjust reagent volumes accordingly for smaller samples.

A. Sample preparation

Infect HEK 293 cells with Adenovirus stock and grow the cells until most show full cytopathic effects. Cells round up and detach.

Harvest the cells by centrifugation. Resuspend the pellet in 10 ml medium but also reserve the remaining medium as it contains significant levels of virus.

Lyse the cells by 3 freeze / thaw cycles. Centrifuge to remove unwanted cellular debris, and then re-combine with the reserved medium.

Digest unwanted nucleic acids by addition of Benzonase® to the supernatant followed by incubation.

Filter the Benzonase® treated supernatant and add 10x Loading Buffer (e.g. 22 ml 10x Loading Buffer to 200 ml culture supernatant).

B. AdenoPACK preparation

Equilibrate the membrane and remove air bubbles from the AdenoPACK units. Use a single unit for up to 60 ml virus culture, or use both units in tandem for up to 200 ml virus culture.



Failure to remove all the air bubbles will reduce the binding of viral particles to the membrane adsorber.

C. Sample loading

Pass the prepared supernatant slowly drop-by-drop through the AdenoPACK units. Use a single unit for up to 60 ml virus culture, or use both units in tandem for up to 200 ml virus culture.



Using the correct flow rate during loading is critical, for maximum binding of viral particles load at no more than 10 ml/min.

D. Washing

Wash away residual culture medium, contaminating proteins and nucleic acids. A higher flow rate may be used for washing.

E. Elution

Elute purified viral particles with Elution Buffer using a special Elution tip to aid in judging the correct flow rate.



Incubation of the AdenoPACK units with Elution Buffer and the correct flow rate during elution is critical, for maximum recovery of viral particles. Elute at no more than 1 ml/min.

F. Final concentration / buffer exchange

Virus concentration may be increased using Vivaspin 20 centrifugal concentrators.

If desired, Vivaspin 20 concentrators may also be used to exchange Elution Buffer for appropriate physiological or storage buffer. (See Usage Tips).

Protocols for operating this kit with laboratory pumps and with a syringe pump are available at: www.vivascience.com/download/pump, www.vivascience.com/download/syringepump

Purification protocol - Techniques

A). Sample preparation

Note: Each AdenoPACK unit may be used singly to purify virus from up to 60 ml culture volume. This kit contains sufficient consumables for two such small preparations.

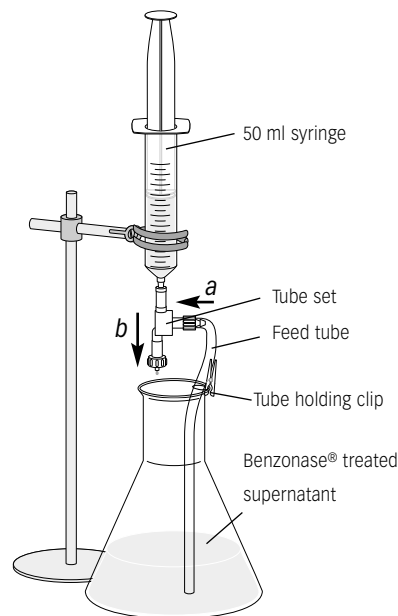
1. Amplify adenovirus in low-passage HEK 293 cells in up to 200 ml total culture volume (E.g. 10x 15 cm plates with 20 ml culture each) that has been infected with an adenovirus stock at an m.o.i. of 10 - 20. **Cultures should be grown in DMEM +10% FBS pH 7.0 - 7.4 at 37°C with 5% CO₂.**
2. Once most of the cells show cytopathic effects (2 - 5 days), pool cells and medium. It may be necessary to detach adhering cells using a pipette or cell scraper.
3. Centrifuge at 3,500 xg for 15 minutes to pellet cells.
4. Decant supernatant to a sterile container and set aside.
5. Re-suspend cell pellet in 10 ml supernatant.
6. Freeze - thaw the cell suspension completely 3 times to disrupt cells alternately using a 25°C water bath and ethanol/dry-ice bath or -80°C freezer.



Caution: Do not allow the temperature to rise above 25°C at any time.

7. Centrifuge at 3,500 xg for 15 minutes to pellet cell debris.
8. Decant viral supernatant, re-combine it with the original supernatant and mix gently.
9. Add 1 µl Benzonase® for each 1 ml culture volume, to a final concentration of 12.5U/ml. E.g. if 200 ml (10x 15 cm plates) were used to cultivate virus, add 200 µl Benzonase®.

10. Mix sample and incubate for 30 min at 37°C in order to digest cellular nucleic acids.
11. Attach the tube set to the 50 ml syringe as shown in the diagram and clamp this to a retort stand.
12. Place the feed tube into the supernatant and draw some 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.



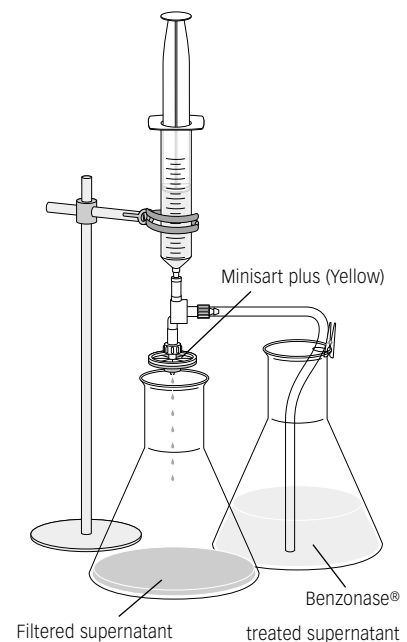
13. Fill the syringe with supernatant and attach a Minisart plus to the syringe assembly.

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



Caution: Once wetted, do not push air through the Minisart during filtration as this may block the filter. If air is drawn into the feed tube, see Troubleshooting.

Note: If the Minisart unit becomes blocked, replace it and continue with the filtration.



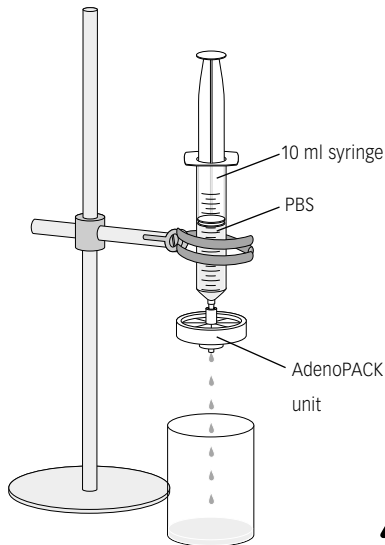
15. Add 1/9 volume of 10 fold Loading Buffer. E.g. 22 ml 10x Loading Buffer to 200 ml cell culture supernatant under agitation.
16. Remove and discard the Minisart.

Purification protocol - Techniques

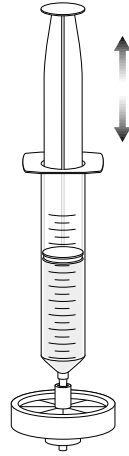
B). AdenoPACK preparation

Note: Air trapped in the AdenoPACK will reduce viral titre. All the air must be removed from the AdenoPACK unit so that virus particles can bind to the membrane.

17. Fill a fresh 10 ml syringe with PBS.
18. Take a single AdenoPACK unit, remove and keep the protective caps and fit to the filled 10 ml syringe as shown below.
19. Apply gentle pressure to the plunger and rinse through 8 - 9 ml of PBS.



20. Pump the syringe plunger gently up and down a few times, to remove air from the AdenoPACK housing.

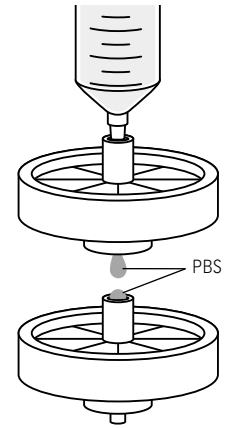


21. Once all the air has been removed from the AdenoPACK housing, continue applying moderate pressure and flush through all except the last 2 ml of PBS.
22. Leave 2 ml PBS in the syringe, replace the cap on the outlet of the filled AdenoPACK unit and remove it from the syringe. Keep upright as much as possible and set it aside.



Caution: Make sure that no air enters the unit.

23. Repeat the process using the second AdenoPACK unit, but do not remove from the syringe. The units need to be connected "wet to wet" to avoid trapping air between the devices. Add some μ l PBS to the inlet of the unit previously prepared and attach it to the outlet of the unit attached to the syringe.



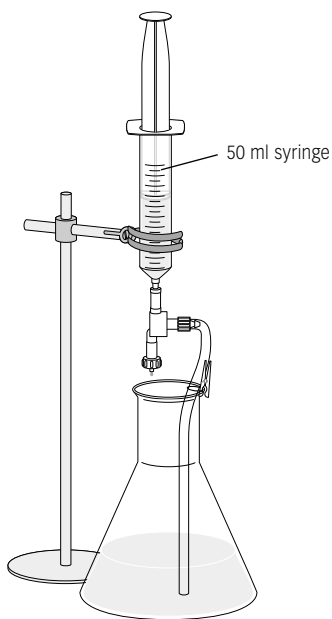
Please note: The outlets of the AdenoPACK units need to be capped before they are removed from the syringe to prevent introducing air into the units.

Purification protocol - Techniques

C). 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.

24. Take the 50 ml syringe and Tube Set previously used for filtration. Place the end of the feed tube into the prepared sample solution. Remove the air from the syringe and valve as before (page 6, No. 12).
25. Fit the wetted AdenoPACK units to the outlet taking care not to trap any air bubbles (wet to wet connection).



26. Pass prepared sample solution slowly through the AdenoPACK units. The optimal flow rate for loading is 10 ml/min; 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 AdenoPACK.

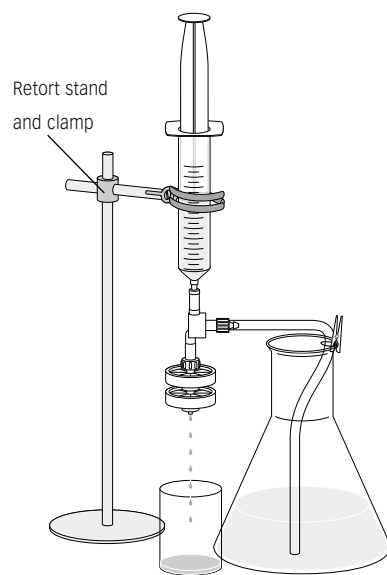


Caution: Press syringe plunger gently. Loading too quickly will reduce the capture of virus particles.

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



Caution: Do not draw air into the feed tube. If this happens, see Troubleshooting.



D). Washing

Note: 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 AdenoPACK to flush the remaining sample solution through before continuing with the main wash.

28. Pour Washing Buffer into the almost empty sample container. Use the same volume as your original culture volume. However Washing Buffer volumes larger than 100 ml are not necessary.
29. Pass the Washing Buffer through the AdenoPACK units. The flow rate for washing may be higher than for loading.



Caution: Do not push air through the AdenoPACK unit during washing.

30. Leave 1 - 2 ml liquid in the syringe at the end to prevent air entering the AdenoPACK and continue to elution step.

Purification protocol - Techniques

E). Elution

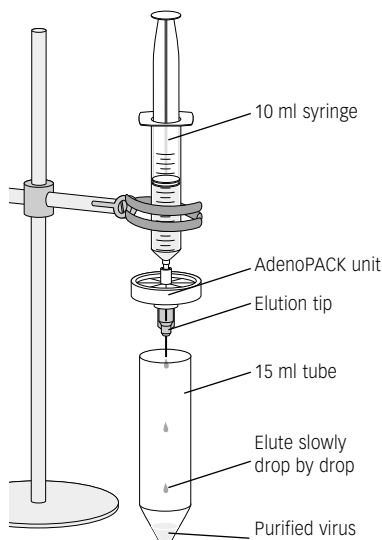
Note: If 2x AdenoPACK units were used in tandem to purify 200 ml virus culture, separate and elute each unit individually. The majority of the virus will be bound to the upper unit.

31. Take a 10 ml syringe and fill with 5 ml Elution Buffer and remove any air bubbles.
32. Detach an AdenoPACK unit from the 50 ml syringe and tube set and attach to the filled 10 ml syringe.
33. Fit the red Elution tip to the outlet of the AdenoPACK unit. This tip makes it easier to obtain the optimum flow rate for elution.
34. Hold the syringe vertically. Taking 1 - 2 minutes, very slowly drop-by-drop pass 1 ml Elution Buffer through the AdenoPACK unit and collect in a sterile 15 ml tube.



Caution: Press syringe plunger very gently, eluting too quickly will reduce the recovery of purified virus. The optimal flow rate for elution is 1 ml/min; you will achieve this if you can count the individual drops.

35. Leave the syringe (with Elution Buffer remaining in it), attached to the AdenoPACK unit and incubate for 5 - 10 min at room temperature.
36. Pass the remaining Elution Buffer through the AdenoPACK unit very slowly as before.
37. Finally using the syringe, push air slowly through the unit to recover as much of the eluate as possible.
38. Elute the second AdenoPACK unit (if used), into the same 15 ml tube, repeating steps 33 to 37.



F). Optional: Final concentration

Note: Further concentrate the viral eluate to increase infectivity. Refer to Vivaspin 20 technical data sheet for detailed operating instructions.

39. Transfer eluate to a Vivaspin 20 centrifugal concentrator 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 centre of the rotor.
40. Centrifuge for 10 min at up to 3,000 xg in a swing-out rotor, or 6,000 xg in a 25° fixed-angle rotor, with cavities accepting 50 ml conical bottom tubes.
41. Check the volume of viral concentrate remaining in the upper chamber and if necessary centrifuge again.



Caution: Do not reduce the volume to less than 1 ml in order to avoid aggregation and loss of infectivity.

42. Recover the concentrated virus by pipette. Resuspend concentrated virus by gently pipetting up and down a few times before recovery.
43. Determine viral titre. Aliquot accordingly and store virus at -80°C.

G). Optional: Buffer exchange

Note: It is sometimes necessary 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 glycerine may take considerably longer to concentrate than the original viral eluate solution; prolong centrifuge times and if necessary use cooling at +4°C.

44. Discard filtrate when sample volume reaches 1 ml, and then add storage / physiological buffer to the concentrate to bring the volume up to 5 ml.
45. Centrifuge again as before and if necessary repeat buffer exchange a second time.
46. Recover the concentrated virus by pipette. Resuspend concentrated virus by gently pipetting up and down a few times before recovery.
47. Determine viral titre. Aliquot accordingly and store virus at -80°C.

Storage buffers for Adenovirus are to be found on page 3 and in the following publication: Hoganson, D. K. et al., Development of a Stable Adenoviral Vector Formulation (2003), Bioprocessing Journal, pp. 43-48.

General information

Typical performance

For a normal yielding vector, 10x 15 cm culture plates purified using this method should yield a range of up to 1×10^{13} viral particles (see table 1.)

Table1: Purification results from preparations with certain Ad5 GFP-constructs - depending on individual conditions values may be different.

Purification method	Process time	Eluate	Recovery**	Viral particles
60 ml culture	1 - 2 hours	1 ml	65%	$1 - 3 \times 10^{12}$
200 ml culture	2 hours	1 ml	80%	1×10^{13}
500 ml CsCl	12 - 48 hours	1 - 2* ml	60 - 70%	10^{11-12}
*after dialysis		**before buffer exchange		

Usage tips

- It is recommended that virus is exchanged into normal physiological buffer before use in tissue culture or cell based assays.
- For culture volumes up to 30 ml, it is not necessary to use the tube set and one-way valve since the whole volume can be contained in the 50 ml syringe.
- Aliquot and store virus at -80°C . Once thawed, keep at $+4^{\circ}\text{C}$ and do not re-freeze.
- Virus should remain viable for up to 2 years at -80°C when purified by this procedure.

Trouble shooting

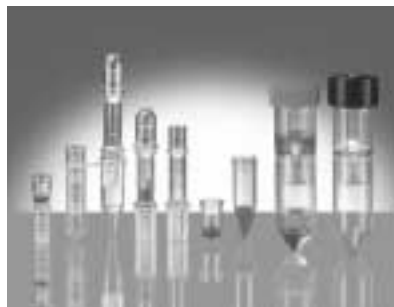
Problem	Cause	Answer
Air in the feed tube	Liquid level low in sample container	Do not expel through the AdenoPACK units. Remove the AdenoPACK unit temporarily from the syringe and expel the air. Re-fill the syringe and tube set with liquid then re-fit AdenoPACK unit
	End of feed tube lifting clear of liquid	Ensure the tube holder is firmly clipped onto the side of the flask
Low virus recovery	Air in the AdenoPACK unit	Avoid trapping air in the AdenoPACK unit
	Flow rate for loading too fast	Load at no more than 10 ml/min
	Flow rate for elution too fast	Elute at no more than 1 ml/min
	Incorrect buffers used	Follow AdenoPACK protocol precisely
	Low viral titre in culture	Optimise virus production
	Buffer left in the AdenoPACK unit	After elution, blow air through the Minisart plus unit to recover all the buffer
Minisart plus clogs during filtration	Air in Minisart plus	Avoid pushing air through the Minisart plus unit once wetted
	Too much residual cellular debris	Centrifuge at 3,500 xg for 15 min to pellet cellular debris prior to final clarification through the Minisart plus
AdenoPACK clogs during filtration	Incomplete clarification of sample	Centrifuge at 3,500 xg for 15 min to pellet cellular debris prior to final clarification through the Minisart plus
The infectivity of the purified Adenovirus particles is lower than expected	The elution tip may in some cases lead to a decrease in virus infectivity due to shearing effects	Elute the virus from the AdenoPACK 100 units without the elution tip. However, pursue the elution dropwise, as recommended in the protocol. Fast elution will lead to lower virus particle yields.

Ordering information

Ordering Information	Description	Pack Size
VS-AVPQ101	Vivapure AdenoPACK™ 100, 200 ml culture volume	1
VS-AVPQ102	Vivapure AdenoPACK™ 100 RT, 200 ml culture volume*	1
VS-AVPQ501	Vivapure AdenoPACK™ 500, 500 ml culture volume	1
Vivascience products in this Kit		
VS2041	Vivaspin 20, 100,000 MWCO PES	12
17829-K	Minisart Plus 0.45 µm CA+GF	50
AdenoPACK 500 Accessories		
VFP001	Masterflex economy drive variable speed peristaltic pump (240 V)	
VFP002	Masterflex economy drive variable speed peristaltic pump (115 V)	
VFP010	Masterflex standard pump head - size 16	
VFP012	Masterflex easy load pump head - size 16	
Related Products		
VS-AVPA001	Pump tubing set for Vivapure AdenoPACK 100	
17829-K	Sartopore 2 150 0.45 - 0.2 µm PES	5

**Kit does not contain Benzonase*

Other products from Vivascience



Vivaspin High Speed Concentrators

Vivaspin high-speed concentrators pioneered the use of vertical-mounted ultrafiltration membranes. The design minimises fouling and significantly increases membrane areas. Now concentrates 3 to 6 times faster without compromising recovery. A graduated concentrate window facilitates operation and sample recovery. Moreover, the range of available membranes and centrifugal devices is the broadest in the industry, allowing a tailored solution to your concentration and desalting applications. For more information, request the Vivascience Ultrafiltration Catalogue.



Vivapure Membrane Adsorbers

Vivapure ion exchange membrane adsorbers are ideal for Prefractionation of cell lysates or other protein samples prior to 2D electrophoresis or chromatography. This ion exchange family of products includes disposable spin columns, 8 well strips, 96 well plates or syringe formats that are compatible with standard laboratory equipment. Unlike chromatography media, membrane adsorbers have large flow-through pores enabling rapid separations, as binding and elution are not diffusion limited. For more information, see Doud et al. *BMG Genomics* 2004, 5:25.

Contact us for more details about Vivapure spin columns

Vivapure IEX Mini	500 µl IEX spin columns
Vivapure IEX Maxi	20 ml IEX spin columns
Vivapure IEX Mega	75 ml IEX spin columns
Vivapure Protein A Mini	500 µl Protein A Affinity spin columns
Vivapure Metal chelate Mini	500 µl metal chelate spin columns

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