Instructions for Use

Vivapure® Adenopack™ 100

Adenovirus (Ad5) purification and concentration kit for up to 200 ml cell culture volume (E.g. 1-10 × 15 cm plate) | For in vitro use only

85030-521-49

SARTORIUS

Vivapure® Adenopack 100 – Introduction

Storage conditions | shelf life
Caution: Benzonase® Nuclease 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.

Introduction
This protocol describes the purification of Adenovirus (Ad5 strains) with Sartobind syringe filters containing an ion exchange membrane adsorber that binds adenoviral particles. Once bound, virus particles can be further purified by washing away nonspecifically 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, Sartobind Q 15 units, centrifugal Vivaspin concentrators and buffers make the following purification procedure as easy as filtration.

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

Vivapure® Adenopack 100

Cat. Number	VS-AVPQ101
Number of purifications possible with Adenopack 100	2×20 - 60 ml or 1×200 ml
Sartobind Q 15 units	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® Nuclease* (12.5 U/µl)	200 µl
Vivaspin 20, 100 kDa MWCO	4
Operating Instructions	1 each for kit and Vivaspin

Materials of construction

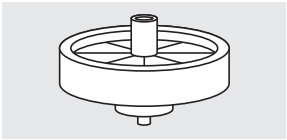
Sartobind Q 15 MA housing	Polysulfone
Minisart housing	Cryolite
Vivaspin 20 housing	Polycarbonate
Vivaspin 20 membrane	PES
Sartobind Q membrane	Stabilised RC
Buffer containers	LDPE
Purification buffers	Proprietary

Kit specifications

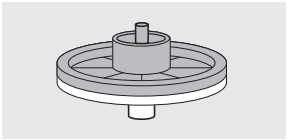
Sample size	20 to 200 ml of Adenovirus supernatants
Virus particles (VP) per ml	Typically up to 1×10^{13}
VP|IU	20 - 50
Processing time	Typically 2 hours
Endotoxin level	<0.025 EU/ml

*Benzonase® Nuclease is manufactured by Merck KGaA. Darmstradt, Germany and is covered by US Patent 5,173,418 qand EP Patent 0,299,866. Nycomed Pharam A/S (Denmark) claims worldwide Patent rights to Benzonase® Nuclease, which are licensed exclusivelt to Merck KGaA. Darmstradt, Germany. Benzonase® is a registered trademak of Merck KGaA. Darmstradt, Germany.

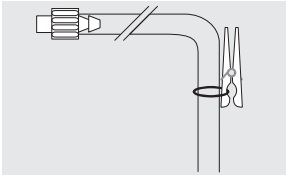
Kit contents



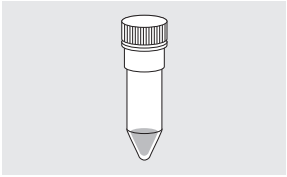
2 × Sartobind Q 15 units with protective end caps



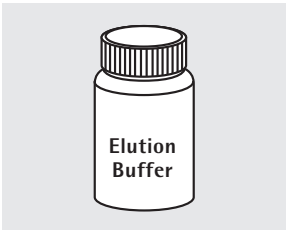
4 × Minisart plus in blister packs (Yellow)



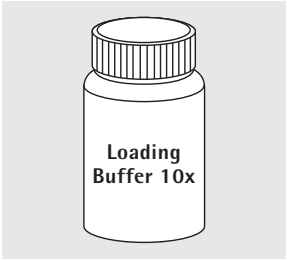
2 × Tube set with one-way valve



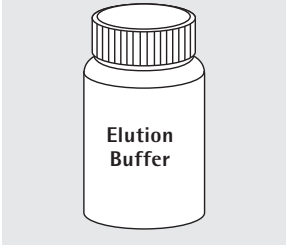
1 × Benzonase® Nuclease stock (12.5 U/µl) Store at -20°C



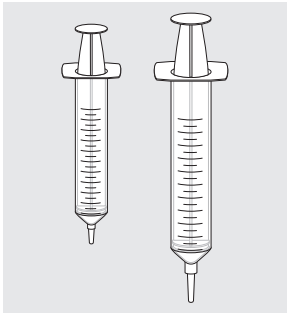
1 × 20 ml Elution Buffer



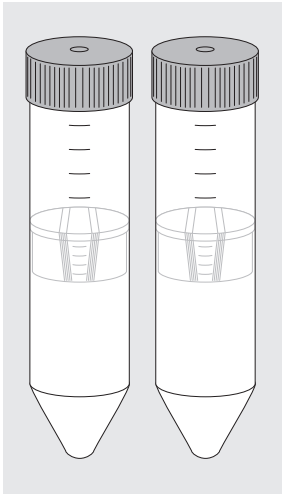
1 × 25 ml Loading Buffer



1 × 120 ml Washing Buffer



2 × 50 ml syringes & 4 × 10 ml syringes

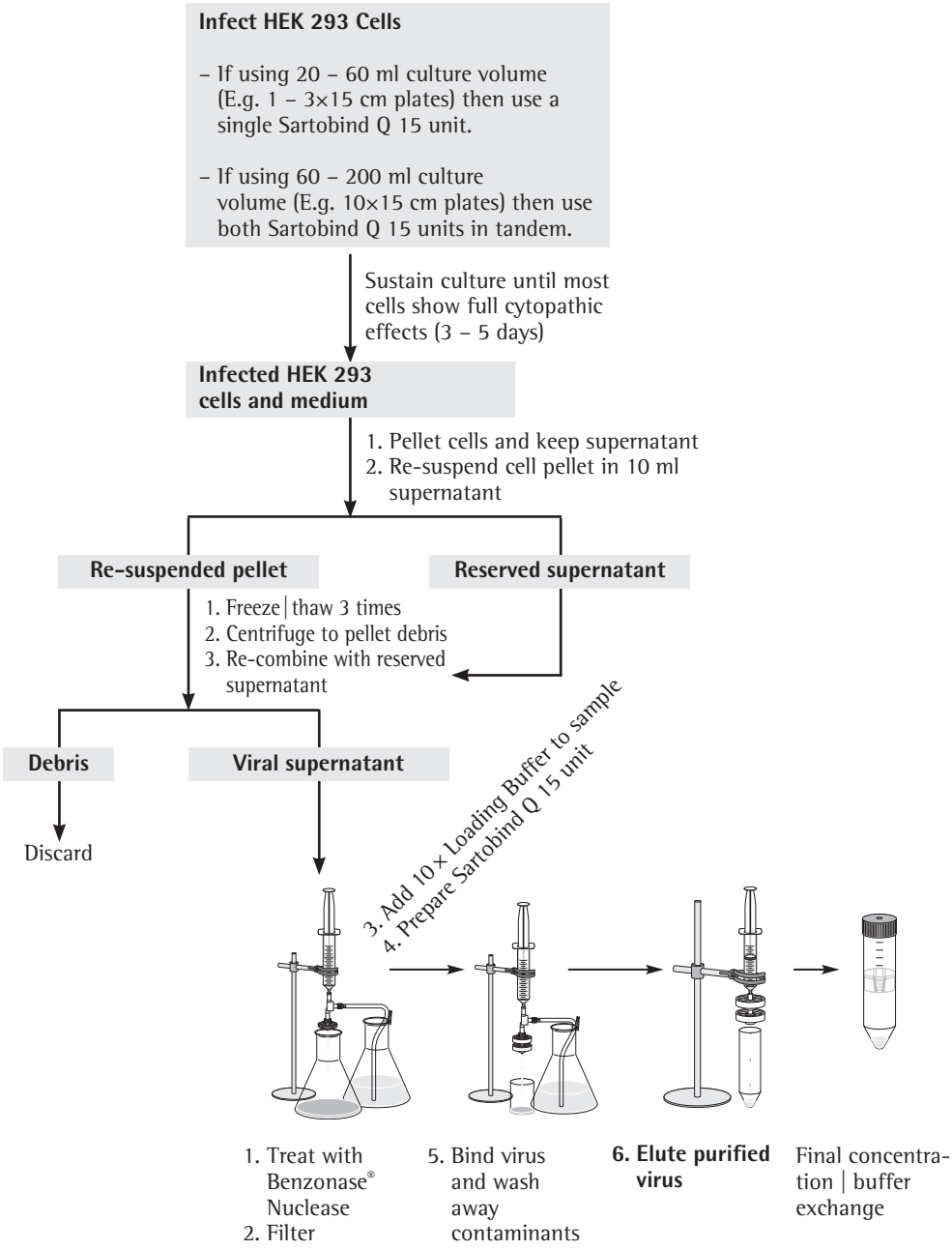


4 × Vivaspin 20 concentrators with 100 kDa membrane

Additional material required but not supplied

Centrifuge with swing-out rotor accepting 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 – Techniques

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

Note: This kit contains sufficient materials for 2×20 – 60 ml preparations or 1×60 – 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® Nuclease to the supernatant followed by incubation.

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

B) Adenopack preparation
Equilibrate the membrane and remove air bubbles from the Sartobind Q 15 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 Sartobind Q 15 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.

Incubation of the Sartobind Q 15 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).

Purification protocol – Techniques

A) Sample preparation
Note: Each Sartobind Q 15 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 lowpassage HEK 293 cells in up to 200 ml total culture volume (E.g. 10×15 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% CO^2.

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® Nuclease for each 1 ml culture volume, to a final concentration of 12.5U/ml. E.g. if 200 ml (10×15 cm plates) were used to cultivate virus, add 200 µl Benzonase® Nuclease.

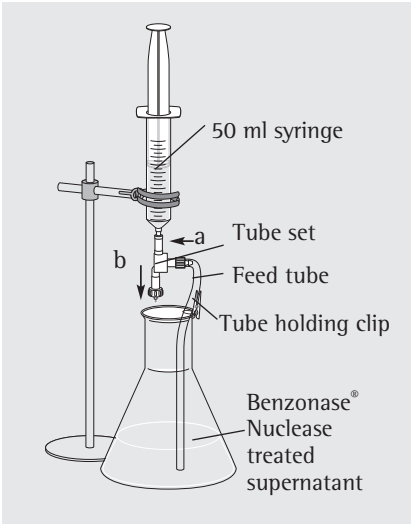
10. Mix sample and incubate for 30 min at 37°C in order to digest cellular nucleic acids.

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).

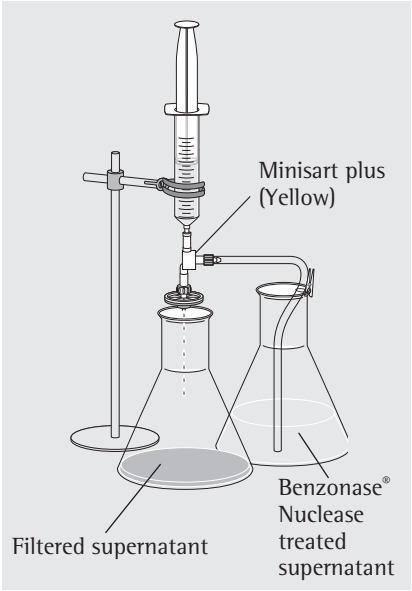


Caution: Once wetted, do not push air through the Minisart during filtration as this may block the filter. If air is drawn into the feed tube, see Troubleshooting.

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 10× Loading Buffer to 200 ml cell culture supernatant. To avoid damage to viral particiles by high salt concentration, add the 10× buffer slowly while swirling the flask of supernatant. It is important to accurately measure the volumes; an incorrect volume of 10× loading buffer will lead to poor binding of viral paritcles.

16. Remove and discard the Minisart.



B) Adenopack preparation
Note: Air trapped in the Sartobind Q 15 will reduce viral titre. All the air must be removed from the Sartobind Q 15 unit so that virus particles can bind to the membrane.

17. Fill a fresh 10 ml syringe with PBS.

18. Take a single Sartobind Q 15 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 5 – 6 ml of PBS.

20. Pump the syringe plunger gently up and down a few times, to remove air from the Sartobind Q 15 housing.

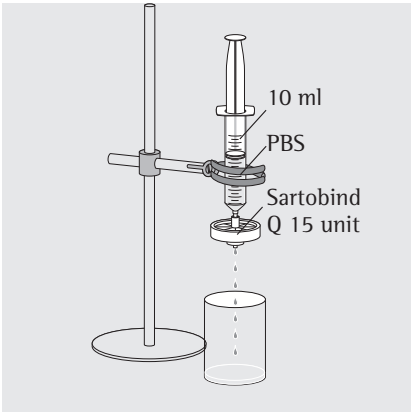
21. Once all the air has been removed from the Sartobind Q 15 housing, continue applying moderate pressure and flush through all except the last 1 ml of PBS.

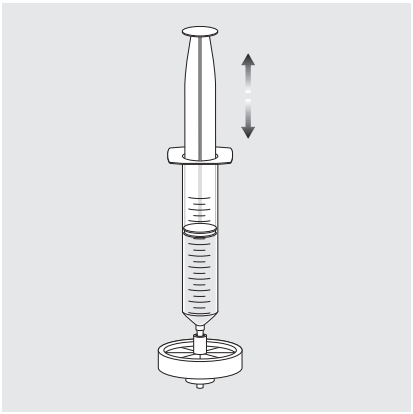
22. Refill the syringe, replace the cap on the outlet of the filled Sartobind Q 15 unit and remove it from the syringe. Keep upright as much as possible and set it aside.

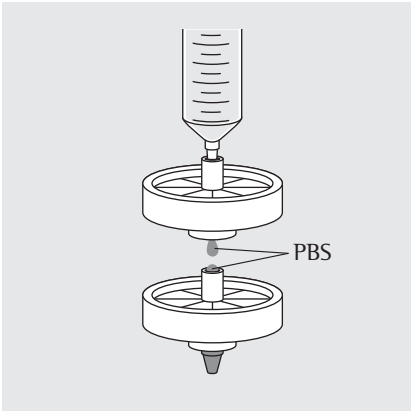
    Caution: Make sure that no air enters the unit.

23. Repeat the process using the second Sartobind Q 15 unit, but do not remove from the syringe in the end. The units need to be connected "wet to wet" to avoid trapping air between the devices. Add some µl 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 Sartobind Q 15 units need to be capped before they are removed from the syringe to prevent introducing air into the units.







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).

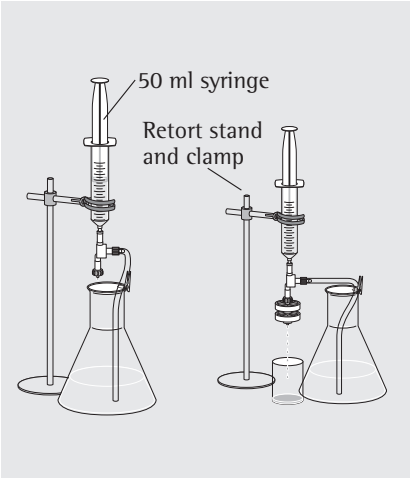
25. Fit the wetted Sartobind Q 15 units to the outlet taking care not to trap any air bubbles (wet to wet connection).

26. Pass prepared sample solution slowly through the Sartobind Q 15 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 Sartobind Q 15 unit.

    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 Sartobind Q 15 unit 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 Sartobind Q 15 units. The flow rate for washing may be higher than for loading.

    Caution: Do not push air through the Sartobind Q 15 unit during washing.

30. Leave 1 – 2 ml liquid in the syringe at the end to prevent air entering the Sartobind Q 15 unit and continue to elution step.

E) Elution

31. Take a 10 ml syringe and fill with 5 ml Elution Buffer and remove any air bubbles.

32. Detach the Sartobind Q 15 units from the 50 ml syringe and tube set and attach to the filled 10 ml syringe.

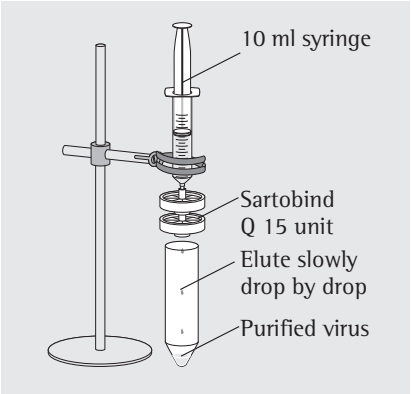
33. Hold the syringe vertically. Taking 1 – 2 minutes, very slowly drop-by-drop pass 1 ml Elution Buffer through the Sartobind Q 15 units and collect in a sterile 15 ml tube.

    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.

34. Leave the syringe (with Elution Buffer remaining in it), attached to the Sartobind Q 15 units and incubate for 5 – 10 min at room temperature.

35. Pass the remaining Elution Buffer through the Sartobind Q 15 units very slowly as before.

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



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

37. 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.

38. Centrifuge for 10 min at up to 800 xg in a swing-out rotor with cavities accepting 50 ml conical bottom tubes.

39. 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.

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

41. Determine viral titre. Aliquot accordingly and store virus at -80°C.

G) Optional: Buffer exchange
Note: It is 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.

42. 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.

43. Centrifuge again as before and if necessary repeat buffer exchange.

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

45. 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, 10×15 cm culture plates purified using this method should yield a range of up to 1×10^{13} viral particles (see table 1.)

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°C and do not re-freeze.
- Virus should remain viable for up to 2 years at - 80°C when purified by this procedure.

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%	$1–3\times10^{12}$
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

Trouble shooting

Problem	Cause	Answer
Air in the feed tube	Liquid level low in sample container	Do not expel through the Sartobind Q 15 units. Remove the Sartobind Q 15 unit temporarily from the syringe and expel the air. Re-fill the syringe and tube set with liquid then re-fit Sartobind Q 15 unit
Air in the feed tube	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 Sartobind Q 15 unit	Avoid trapping air in the Sartobind Q 15 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 Sartobind Q 15 unit	After elution, blow air through the Minisart plus unit to recover all the buffer
Low virus recovery	Virus cultures allowed to grow too long may result in decreasing titres	Harvest when cytopathic effects are obvious in the majority of cells
Minisart plus clogs during filtration	Air in Minisart plus	Avoid pushing air through the Minisart plus unit once wetted
Minisart plus clogs during filtration	Too much residual cellular debris	Centrifuge at 3,500 × g for 15 min to pellet cellular debris prior to final clarification through the Minisart plus
Sartobind Q 15 unit clogs during filtration	Incomplete clarification of sample	Centrifuge at 3,500 × g for 15 min to pellet cellular debris prior to final clarification through the Minisart plus
The infectivity of the Adenovirus particles is lower than expected	Elution buffer not exchanged 10× Loading buffer added to quickly or without stirring	Exchange purified virus into purified physiological buffer To avoid damage to viral particles by locally high salt concentration, add the 10× buffer slowly while stirring the flask of supernatant

Ordering information

Ordering Information		Pack Size
VS-AVPQ020	Vivapure® Adenopack™ 20, 20 ml culture volume	6
VS-AVPQ022	Vivapure® Adenopack™ 20 RT, 20 ml culture volume*	6
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
VS-AVPQ502	Vivapure® Adenopack™ 500RT, 500 ml culture volume*	1

Sartorius Biotech Biolab products in this Kit		
VS2041	Vivaspin 20, 100,000 MWCO PES	12
17829-K	Minisart Plus 0.45 µm CA+GF	50
Q15X	Sartobind Q 15	2

Adenopack 500 Accessories		
VFP001	Masterflex economy drive variable speed peristaltic pump (240 V)	
VFP002	Masterflex economy drive variable speed peristaltic pump (115 V)	
VFA012	Masterflex easy load pump head – size 16	

Related Products		
VS-AVPA001	Pump tubing set for Vivapure® Adenopack 100	
5441307H0-00	Sartopore 2 150 0.45 – 0.2 µm PES 5	

* Kit does not contain Benzonase® Nuclease

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The information and figures contained in these instructions correspond to the version date specified below.
Sartorius reserves the right to make changes to the technology, features, specifications and design of the equipment without notice.
Masculine or feminine forms are used to facilitate legibility in these instructions and always simultaneously denote the other gender as well.

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