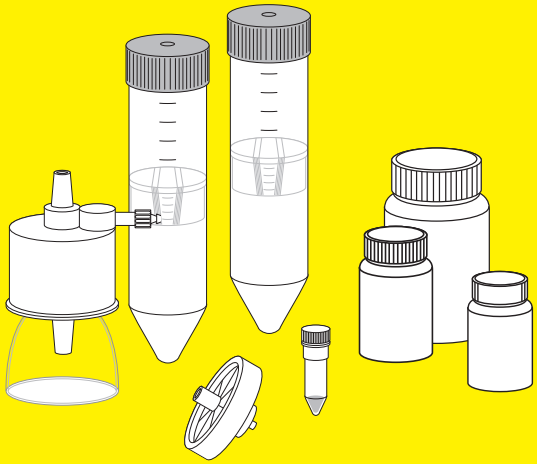
Instructions for Use

Vivapure® Adenopack™ 500

Adenovirus (Ad5) purification and concentration kit for up to 500 ml cell culture volume (E.g. 5 - 25 × 15 cm plates) | For in vitro use only



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Vivapure Adenopack 500 – 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.

Introduction
This protocol describes the purification of Adenovirus (Ad5 strains) with Sartobind Q 75 syringe filters containing a membrane adsorber that selectively 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 75 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.

PROGEN Biotechnik

Vivapure Adenopack 500

Cat. Number	VS-AVPQ501
Sartobind Q 75 unit	1
Sartopore 2 150 clarifying filter	1
10 ml syringe	1
Tubing set	2
Loading Buffer (10×)	60 ml
Washing Buffer (10×)	30 ml
Elution Buffer (1×)	20 ml
Benzonase®* (12.5 U/µl)	500 µl
Vivaspin 20, 100 kDa MWCO	2
Technical data sheet	1 each for Kit and Vivaspin

Materials of construction

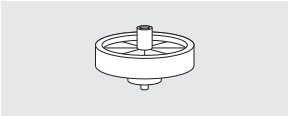
Sartobind Q 75 MA housing	Polysulfone
Sartopore 2 150 clarifying filter housing	Polypropylene
Sartobind Q membrane	Stabilised RC
Buffer containers	LDPE
Purification buffers	Proprietary

Kit specifications

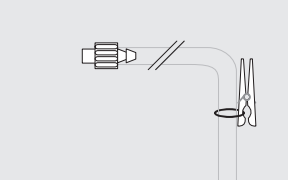
Sample size	Up to 500 ml of Adenovirus supernatants
Virus particles (VP)	Typically up to 1 – 3 × 10^{13}
VP/IU	20–50
Processing time	Typically 2 hours

* Benzonase® Nuclease is manufactured by Merck KGaA, Darmstadt, 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, Darmstadt, Germany. Benzonase® is a registered trademark of Merck KGaA, Darmstadt, Germany.

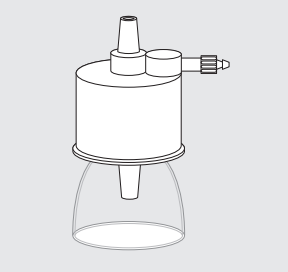
Kit contents



Sartobind Q 75 unit
with protective end caps



2× Pump tube set (Luer connector fits both Sartobind Q 75 and Sartopore units)



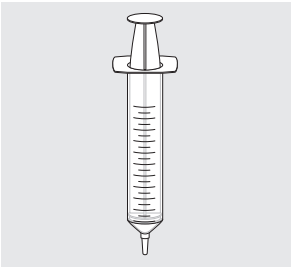
1× Sartopore 2
150 clarifying filter

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

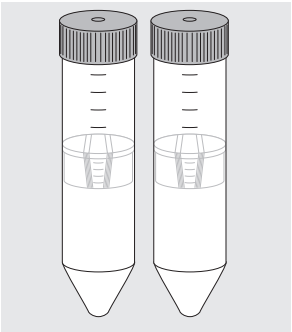
60 ml Loading Buffer (10×)

30 ml Washing Buffer (10×)

20 ml Elution Buffer



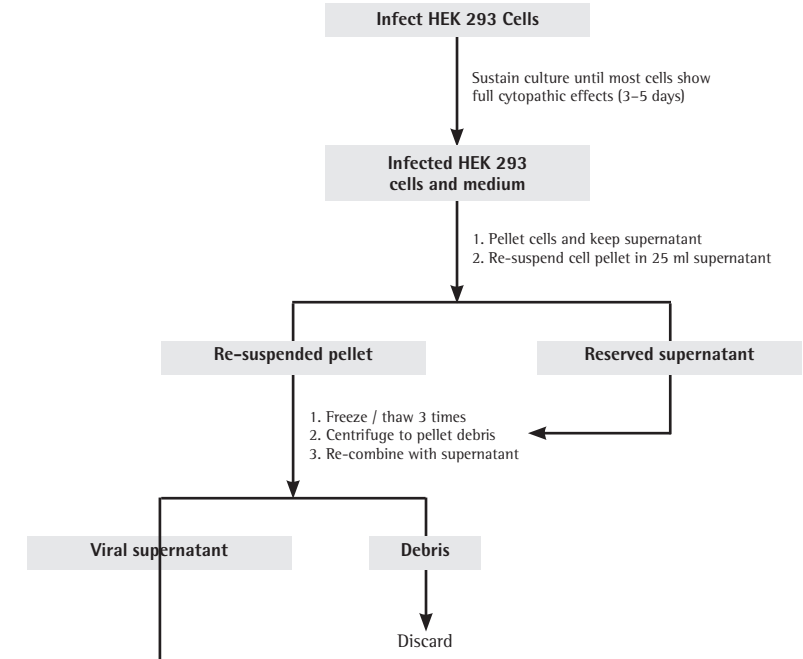
10 ml syringe

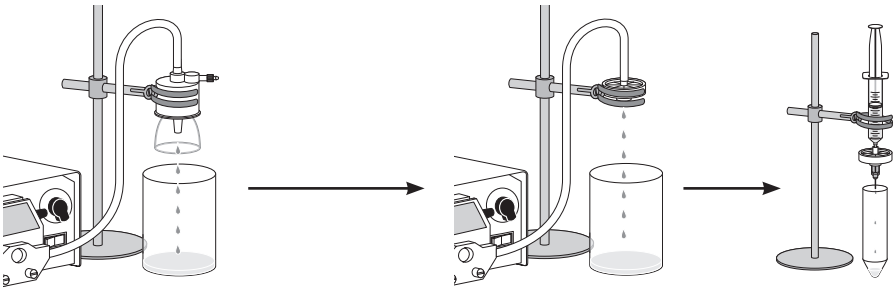


2× Vivaspin 20 concentrators with 100 kDa MWCO PES membrane

Additional material required but not supplied
Centrifuge with rotor accepting 50 ml falcon tubes
Peristaltic pump accepting Masterflex L/S 16 size tube
Retort stand and clamp
100 ml measuring cylinder
Timer or Stopwatch
300 ml Phosphate buffered Saline pH 7.4 (PBS)
Ethanol|dry ice bath or –80 °C freezer
Water bath at 25 °C
Sterile plastic container for sample handling
Sterile 15 ml 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





1. Treat with Benzonase®
2. Filter

3. Add Loading Buffer
4. Prepare Sartobind Q 75 unit

5. Bind virus and wash away contaminants

6. Elute purified virus
7. Concentrate further or exchange buffer using Vivaspin

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 to concentrate and purify virus from 500 ml culture medium. The detailed protocols are written as though for a 500 ml preparation, please adjust reagent volumes accordingly for smaller samples.

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

Sample preparation
Harvest cells by centrifugation. Resuspend the pellet in 25 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 the addition of Benzonase® to the supernatant followed by incubation.

Filter the Benzonase® treated supernatant and add 10× Loading Buffer. Volume of supernatant ÷ 9 = volume of 10× Loading Buffer to add.

Sartobind Q 75 preparation
Equilibrate the membrane and remove air bubbles from the Sartobind Q 75 unit before loading virus. Failure to remove all the air bubbles will reduce the binding of virus to the membrane adsorber.

Sample loading
Pass the prepared supernatant slowly drop-by-drop through the Sartobind Q 75 unit. Using the correct flow rate is critical, for maximum binding of virus load at no more than 10 ml/min.

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

Elution
Elute purified viral particles by passing Elution Buffer through the Sartobind Q 75 unit with a syringe. Incubation of the Sartobind Q 75 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/min.

Final concentration|buffer exchange virus concentration may be increased using the Vivaspin 20 concentrators supplied with this kit.

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

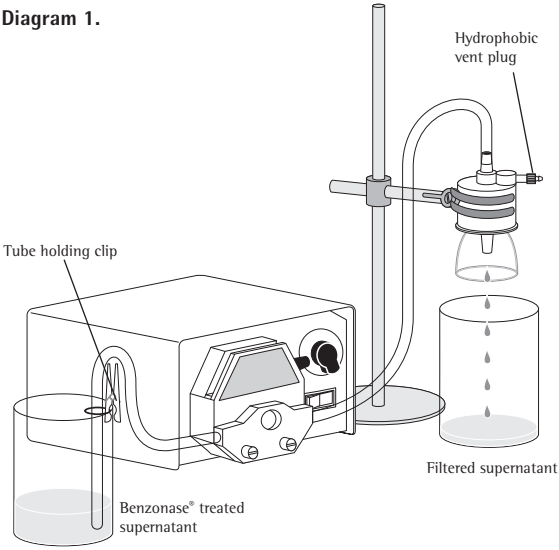
A). Virus culture
Note: The 10 × Loading Buffer included in the Vivapure Adenopack 500 kit is specially formulated to be used with the specified culture conditions, please follow them carefully.

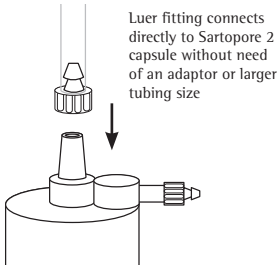
Seed up to 25× 15 cm plates with HEK 293 cells in DMEM with 10% FBS, pH 7.0–7.4. When the cell monolayer reaches a confluency of 60–80%, infect cells with an Ad5 type adenovirus stock at an m.o.i. of at least 20. Culture infected cells at 37°C with 5% CO_2 for 3–5 days until most cells show cytopathic effects. Cells should round up and detach.

Sample preparation

1. Pool infected cells and medium. Pellet cells by centrifugation at 3,500 × g for 15 min.
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.
5. Pellet debris by centrifugation at 3,500 × g for 15 min. Decant supernatant and add to the reserved supernatant from step 2.
6. Add 1 µl Benzonase® for each 1 ml supernatant, to a final concentration of 12.5 U/ml. (E.g. to 500 ml supernatant, add 500 µl Benzonase®). Mix sample and incubate at 37°C for 30 min.
7. Dilute 10 × Washing Buffer to working concentration by adding to 270 ml deionised water and mixing well. Set aside until later.
8. Assemble equipment as shown in diagram 1. Loosen the hydrophobic vent plug in the Sartopore 2 casing. Pump the Benzonase® treated supernatant through the filter at 10–20 ml/min. 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.
9. Add 10 × Loading Buffer to Benzonase® treated supernatant slowly under agitation. Volume of supernatant ÷ 9 = volume of 10 × Loading Buffer to add. E.g. to 500 ml supernatant add 56 ml 10 × Loading Buffer.

Diagram 1.





Purification protocol – Techniques

B). Sartobind Q 75 preparation
Note: Air trapped in the Sartobind Q 75 will reduce viral titre. All the air must be removed from the Sartobind Q 75 unit so that virus particles can bind to the membrane.

10. Using a fresh tube set, assemble the equipment as set out in diagram 2.

11. Pour 300 ml PBS into the beaker.

12. Pump PBS through the Sartobind Q 75 unit to ensure it is fully wetted.

13. Adjust the pump setting until a flow rate of 10 ml/min is achieved.

14. Pump through 30–50 ml PBS then stop the pump using the switch; do not adjust the pump speed. Caution: Loading too quickly will reduce the capture of virus particles and may result in decreased viral titre.

Sample Loading|washing

15. Remove the feed tube from the beaker containing PBS and place into the container of prepared viral supernatant. Pump through the Sartobind Q 75 500 unit at the set speed of 10 ml/min. Collect flowthrough and treat as biohazard waste.

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

17. Pump the Washing Buffer through the filter at 10–20 ml/min.

Diagram 2.

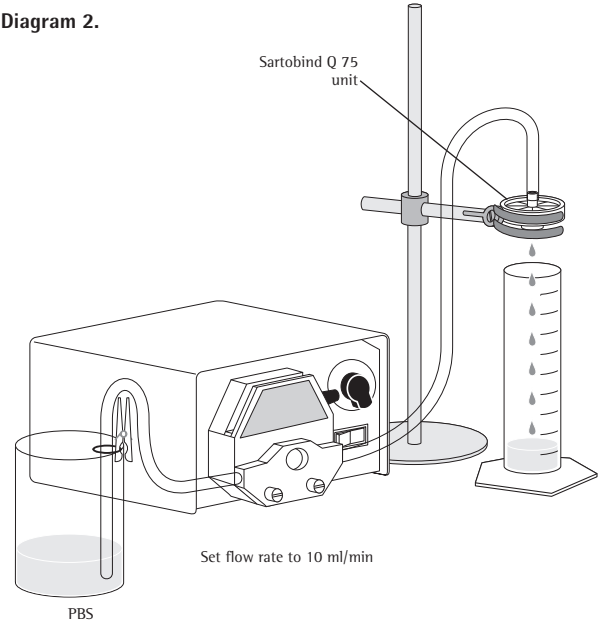
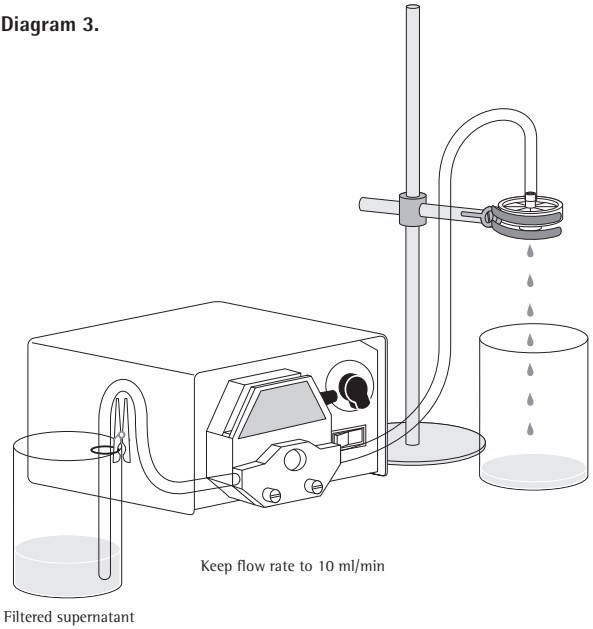


Diagram 3.



Purification protocol – Techniques

Elution

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

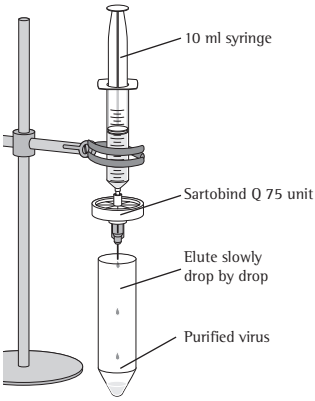
19. Detach the Sartobind Q 75 unit from the tubing assembly and attach the filled 10 ml syringe to the inlet.

20. Hold the syringe vertically. Very slowly (drop-by-drop) pass 1 ml Elution Buffer through the Sartobind Q 75 unit and collect in a sterile 15 ml tube. (See diagram 4.) 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.

21. Leave the syringe (with the remaining 9 ml Elution Buffer in it), attached to the Sartobind Q 75 unit and incubate for 5–10 min at room temperature.

22. Pass the remaining Elution Buffer through the Sartobind Q 75 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 20–22.

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



Final Concentration|Buffer Exchange

Note: Further concentrate the viral eluate to increase infectivity. Refer to Vivaspin 20 technical data sheet for detailed operating instructions. 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; prolong centrifuge times. Cooling at +4°C is highly recommended.

24. 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 center of the rotor.

25. Centrifuge for 15 min 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.

26. 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 virus aggregation and loss of infectivity.

27. Discard filtrate when sample volume reaches 1 ml, and then add 4 ml of storage|physiological buffer (E.g. 20 mM Tris/HCl, 25 mM NaCl, 2.5% Glycerol (w/v), pH 8.0) to the concentrate to bring the volume up to 5 ml. This will bring the virus to normal physiological conditions.

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

29. Determine viral titer. Aliquot and store virus at –80°C.

General information

Typical performance
For a normal yielding vector, 25 × 15 cm culture plates purified using this method should yield a range of up to $1–3 \times 10^{13}$ 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.

- 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
500 ml culture	1–2 hours	1 ml	80%	$1–3 \times 10^{13}$
500 ml CsCl	12–48 hours	1–2 ml	60–70%	1×10^{13}

* after buffer exchange
** 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 75 units. Remove the Sartobind Q 75 unit temporarily from the syringe and expel the air. Re-fill the syringe and tube set with liquid then re-fit Sartobind Q 75 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 100 unit	Avoid trapping air in the Sartobind Q 75 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 75 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
Sartopore 2 150 clogs during filtration	Air trapped in Sartopore housing	Loosen hydrophobic vent plug to allow air bubbles to escape
Sartopore 2 150 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 Sartopore 2 150
Sartobind Q 75 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 Sartopore 2 150

Ordering information

Ordering Information	Description	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 products in this kit		
VS2041	Vivaspin 20, 100,000 MWCO PES	12
5441307H0-00	Sartopore 2 150 0.45–0.2 µm PES	5
Q75X	Sartobind Q 75	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	
17829-K	Minisart Plus 0.45 µm CA+GF	50

* 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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