Instructions for Use

Vivapure® Adenopack™ 20

Adenovirus (Ad5) purification and concentration kit for up to 20 ml cell culture volume (E.g. 1×15 cm plate) | For in vitro use only

85030-523-69

SARTORIUS

Vivapure Adenopack 20 – 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 Vivapure Q Maxi M spin columns 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 one hour.

The Vivapure Q Maxi M is designed for parallel purification and concentration of Ad5 strain adenovirus from 20 ml culture medium.

In contrast, traditional CsCl gradient centrifugation is a time consuming method, typically taking 1–2 days, which can only be performed efficiently for cell culture volumes larger than 100 ml. Furthermore, the toxicity of the media places limitations on downstream applications.

Ready to use filter devices, Vivapure Q Maxi M spin columns, 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

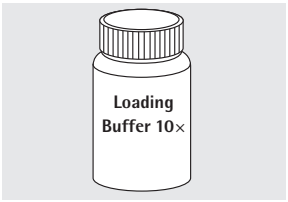
Vivapure Adenopack 20	
Cat. Number	VS-AVPQ020
Number of purifications possible with Adenopack 20	6×20 ml
Vivapure Q Maxi M spin columns	6
Vivaclear Maxi 0.45 µm PES	6
Empty 50 ml tubes	6
Loading Buffer (10×)	25 ml
Washing Buffer (10×)	30 ml
Elution Buffer	20 ml
Benzonase®* (12.5 U/µl)	120 µl
Vivaspin 20, 100 kDa MWCO	6
Technical data sheet	1 each for kit and Vivaspin

Materials of construction	
Vivapure Q Maxi M spin column housing	Polypropylene
Vivaclear Maxi housing	Polypropylene
Vivapure Q membrane	Stabilised RC
Buffer containers	LDPE
Purification buffers	Proprietary

Kit specifications	
Sample size	20 ml of Adenovirus supernatants
Virus particles (VP) per ml	Typically up to 1×10^{11}–10^{12}
VP/IU	50–100
Processing time	Typically 1 hour
Endotoxin level	<0.025 EU/ml

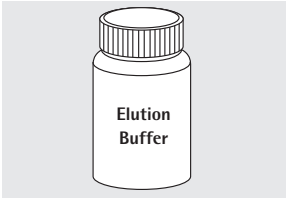
* Benzonase® Nuclease is manufactured by Merck KGaA, Darmstadt, Germany and is covered by US Patent 5,173,418 and EP Patent 0,229,866. Nycomed Pharma A/S (Denmark) claims worldwide patent rights to Benzonase® Nuclease, which are licensed exclusively to Merck KGaA, Darmstadt, Germany. Benzonase® is a registered trademark of Merck KGaA, Darmstadt, Germany.

Kit contents



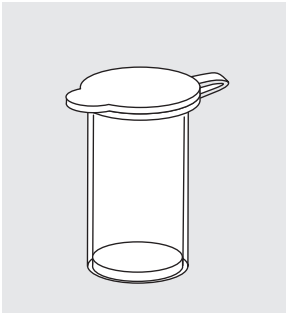
1× 25 ml Loading Buffer

1× 30 ml Washing Buffer

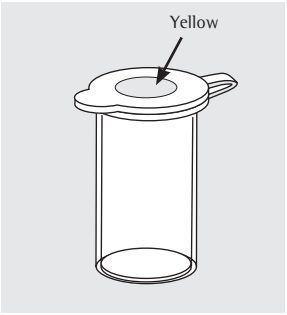


1× 20 ml Elution Buffer

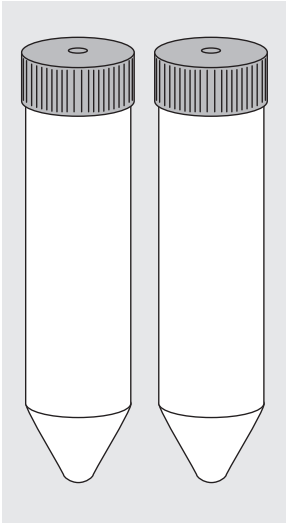
1× Benzonase® stock (12.5 U/µl)
Store at -20°C



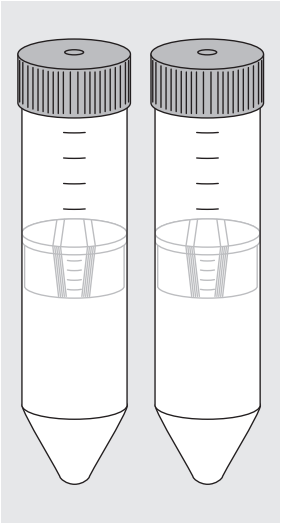
6× Vivapure Q Maxi M spin columns



6× Vivaclear Maxi spin columns



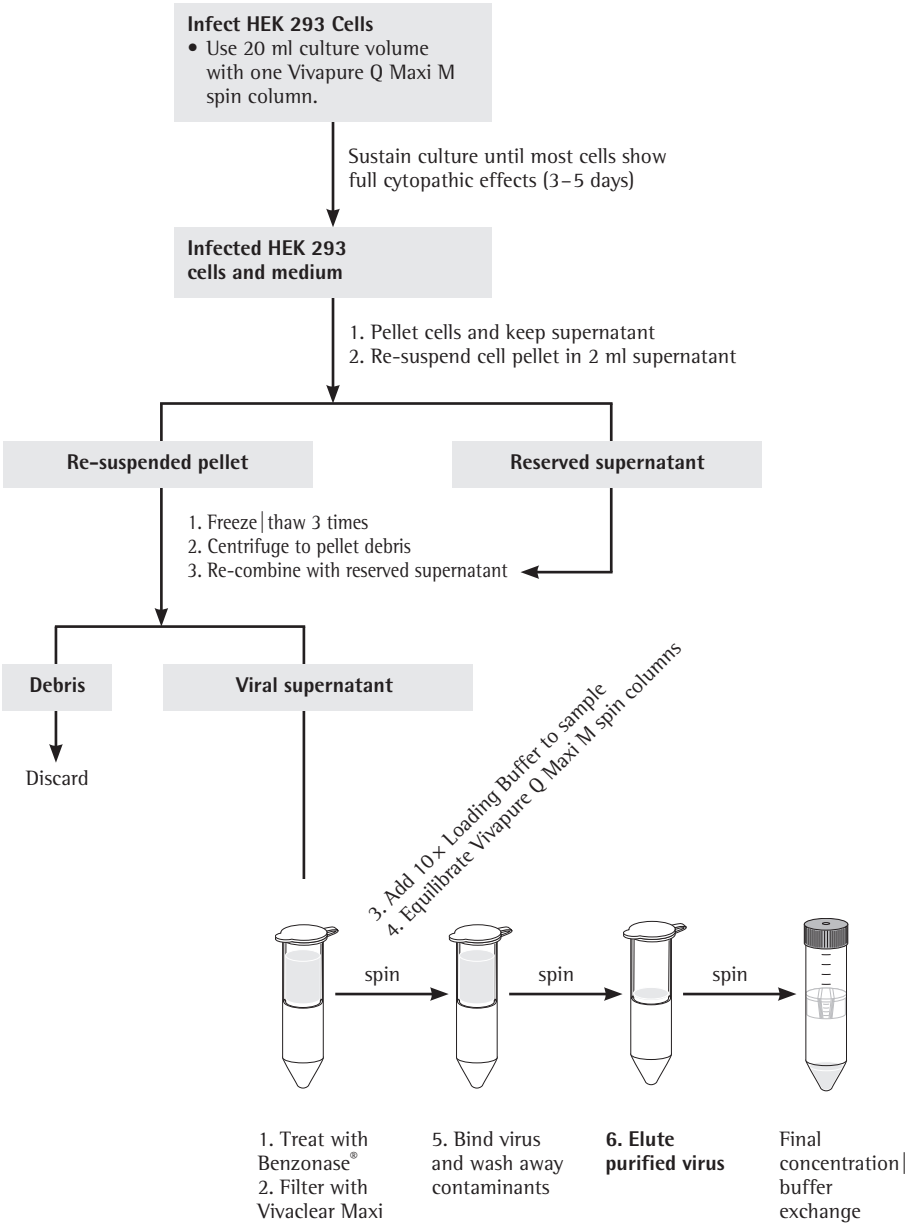
6× 50 ml tubes with screw caps



6× Vivaspin 20 concentrators with 100 kDa membrane

Additional material required but not supplied
Centrifuge with swing out rotors accepting 50 ml falcon tubes
Ethanol | dry ice bath or -80°C freezer
Water bath at 25°C
Sterile plastic container for sample handling
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

A). Sample preparation
Note:
Each Vivapure Q Maxi M spin column may be used to purify virus from up to 20 ml culture volume. This kit contains sufficient consumables for six such small preparations. Please adjust reagent volumes accordingly for smaller samples.

1. Amplify adenovirus in low-passage HEK 293 cells in up to 20 ml total culture volume (E.g. one 15 cm plate with 20 ml culture) 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 × g for 15 minutes to pellet cells.

4. Decant supernatant to a sterile container and set aside.

5. Re-suspend cell pellet in 2 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.

7. Centrifuge at 3,500 × g 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 12.5 U/ml. E.g. if 20 ml (1 × 15 cm plates) were used to cultivate virus, add 20 µl Benzonase®.

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

11. Load digested supernatant on a Vivaclear Maxi and spin the device 5 minutes at 500 × g or until the whole volume has passed the membrane.

12. Collect the flow-through. Estimate the volume and slowly add 1/9 volume of 10 fold loading buffer under agitation to avoid osmotic shock in the virus particles. E.g. 2 ml to 18 ml flow-through. Accurate volume measurements of the supernatant and 10 × Loading Buffer are critical to achieving the right conditions for binding virus particles.

B). Vivapure Q Maxi M preparation
13. Dilute 10 x Washing Buffer to working concentration, e.g. for one preparation: 5 ml buffer with 45 ml deionised water, and mix well.

14. Equilibrate the Vivapure Q Maxi M spin column with 5 ml diluted Washing Buffer and spin for 5 minutes at 500 × g. We recommend the use of a swing-out rotor to ensure the uniform flow of your sample solution.

C.) Adenovirus purification
15. Load the sample (no more than 20 ml) into the Vivapure Q Maxi M insert and spin the device for 5 min at 500 × g or until the whole volume has passed the membrane. Collect flow-through and repeat the step with residual sample if necessary.

16. Wash the spin column with 18 ml Washing Buffer by spinning for 5 min at 500 × g. Remove flow-through and repeat the wash step once.

17. Using a fresh collection tube, elute adenovirus off the Vivapure Q Maxi M membrane using 1 ml Elution Buffer. Pipette buffer onto the membrane, centrifuge for 30 s at 500 × g and incubate 10 min. Then spin the device for 5 min at 500 × g and collect the Adenovirus-containing eluate. Applying a second elution step could improve yield but will dilute virus titre.

Purification protocol – Techniques

D). Optional: Buffer exchange and further concentration
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.

18. Transfer eluate to a Vivaspin 20 centrifugal concentrator and add storage | physiological buffer to the concentrate to bring the volume up to 10 ml. 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.

19. Centrifuge for 30 min at up to 800 × g in a swing-out rotor, or 25° fixed-angle rotor, with cavities accepting 50 ml conical bottom tubes.

20. Check the volume of viral concentrate remaining in the upper chamber and if necessary centrifuge again and repeat buffer exchange a second time.

⚠ **Caution:**
Do not reduce the volume to less than 200 µl in order to avoid aggregation and loss of infectivity.

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

22. 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, 1×15 cm culture plates purified using this method should yield a range of up to 1×10^{12} 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 parti-cles
20 ml culture	1 hour	1 ml	65–70%	$1\times10^{11-12}$
500 ml CsCl	12–48 hours	1–2* ml	60–70%	$1\times10^{11-12}$

* after dialysis
** before buffer exchange

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
Additional products in this Kit		
VS2041	Vivaspin 20, 100,000 MWCO PES	6
Related Products		
5441307H0-00	Sartopore 2 150 0.45–0.2 µm PES	5
17829-K	Minisart Plus 0.45 µm CA + GF	50

* Kit does not contain Benzonase®

Sartorius Stedim Lab Ltd.
Sperry Way, Stonehouse Park
GL10 3UT Stonehouse, Gloucestershire, UK

Phone: +44 1453 821972
www.sartorius.com

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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