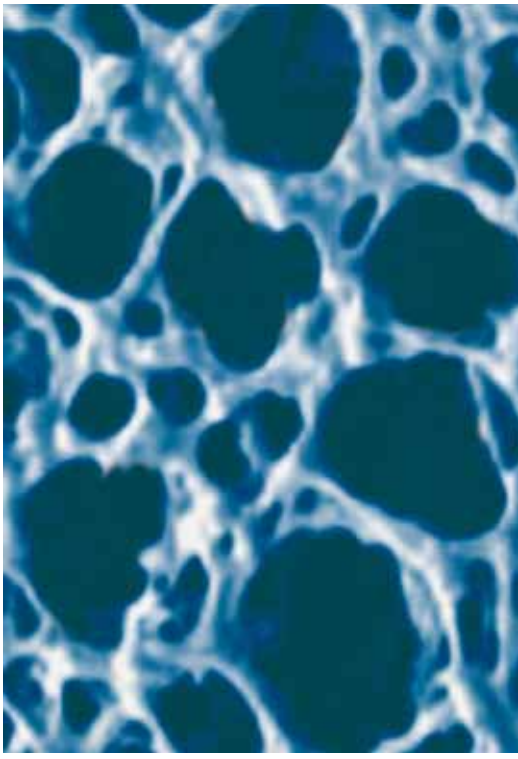
sartorius stedim biotech

Vivapure®
Virus Purification and Concentration Kits



turning science into solutions

Fast and easy to use kits for virus purification

Recombinant virus vectors are the preferred method for a wide range of gene delivery applications. Especially **adenovirus type 5** and **VSV-G pseudotyped lentivirus** are two frequently utilized viral vectors for in vitro and in vivo applications.

Recombinant adenovirus vectors are versatile tools in research and therapeutic applications for gene transfer and protein expression in cell lines that have low transfection efficiency with liposomes. After entering cells, the virus remains epichromosomal (i.e. does not integrate into the host chromosome, leaving the host genome unaffected). The delivery of RNAi into cells is becoming a major application for adenovirus vectors.

Lentivirus vectors are frequently used in gene transfer studies, due to their ability of gene transfer and integration into dividing and non-dividing cells. The pseudotyped envelope with vesicular stomatitis virus envelope G (VSV-G) protein broadens their target cell range. Lentiviral vectors have been shown to deliver genes into cell types (e.g. neurons, lymphocytes and macrophages) which other retrovirus vectors could not be used for. The lentivirus vector is increasingly used to integrate siRNA efficiently in a wide variety of cell lines and primary cells, both in vitro and in vivo.

Rapid virus purification by Membrane Chromatography

The Sartobind® ion exchange membrane adsorber technology used in AdenoPACK and LentiSELECT is unique in its capability to efficiently and rapidly capture and recover large virus particles. When compared to chromatography media, membrane adsorbers provide large 3000 nm pores allowing unrestricted access and recovery of virus from the charged adsorber surface. Convective flow through the syringe filter devices provides high-speed separations not possible with traditional chromatography, cesium chloride density gradients and ultracentrifugation methods. Our membrane adsorbers with porous matrices, high capacities, low differential pressures, high flow rates and low unspecific adsorption show an excellent performance in small scale virus purification. Additionally, they are also scalable and confirm to cGMP facilities to large volume, high performance separation, reducing the processing time by a factor of 10 in the final process.

Adenovirus Purification with AdenoPACK kits

AdenoPACK 20|100|500
The AdenoPACK adenovirus purification and concentration kits offer researchers who need to recover up to 3×10^{13} purified recombinant adenovirus particles for in-vitro transfection a fast, safe and easy to use solution. The kits include all reagents and devices necessary for clarification, purification and concentration of adenovirus type 5 from HEK293 cell cultures in only two hours. These straight forward kits replace time-consuming and labor-intensive 48 hour CsCl density gradients.

AdenoPACK kits are offered as AdenoPACK 20, AdenoPACK 100 and AdenoPACK 500, for the purification and concentration of adenovirus type 5 from 20 ml to 500 ml cell culture, leading to 1×10^{11}- 3×10^{13} purified viral particles. For each sample volume, the most convenient handling method is offered for ultimate convenience.

To this end, preparations using AdenoPACK 20 are pursued in spin column format in a centrifuge, AdenoPACK 100 is a manually operated kit in syringe filter format*, and AdenoPACK 500 is a pump driven kit.

* Vivapure® AdenoPACK 100 can optionally be operated with a laboratory pump and an infusion pump, for which protocols are provided on our web page www.sartorius-stedim.com. Additionally, the tubes and adaptors needed for these operation modes can be ordered.

AdenoPACK advantages

Fast and easy virus purification
- Purification completed in 2 hours
- Convenient, over 10 x faster alternative to CsCl density gradient

Quantitative yields
- In contrast to CsCl density gradient, the complete cell culture is used for virus purification and not only the viral pellet

Flexible product range
- Applicable from initial construct screening to large scale virus production

Complete Kit
- Including filtration devices, AdenoPACK units for virus purification, Vivaspin and all buffers

Low endotoxin levels
- High cell viability and infection rates due to endotoxin levels of < 0.025 EU/ml

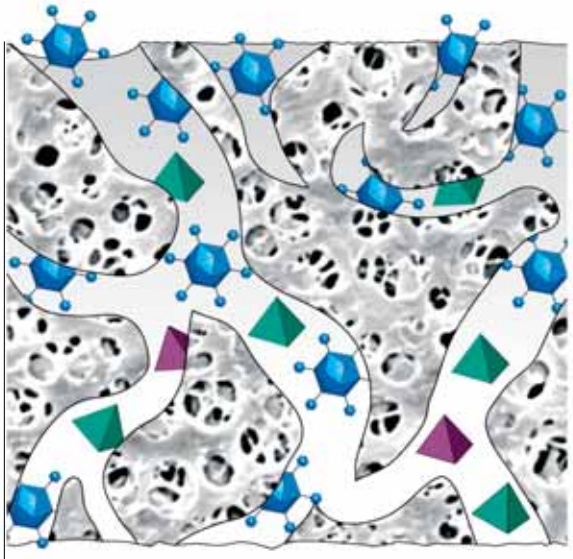
Purification results from preparations with Ad5 GFP-constructs

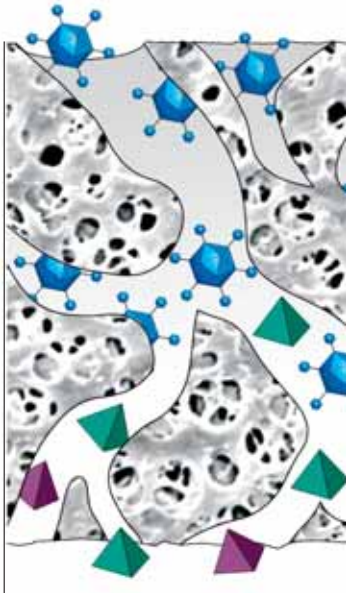
Purification method	Process time	Eluate	Recovery***	Viral Particles
AdenoPACK 20|20 ml culture	1 hour	1 ml	65–70%	$1 \times 10^{11-12}$
AdenoPACK 100|60 ml culture	1–2 hours	1 ml	65%	$1–3 \times 10^{12}$
AdenoPACK 100|200 ml culture	2 hours	1 ml	80%	1×10^{13}
AdenoPACK 500|500 ml culture	2 hours	1 ml	80%	$1–3 \times 10^{13}$
500 ml CsCl density gradient	24–48 hours	1–2 ml**	60–70%	$1 \times 10^{11-12}$

**after dialysis
***before buffer exchange

Sample Preparation 45 min. **Sample Loading 30 min.** **Washing 10 min.**

Easy to follow and fast protocol for virus purification. The Vivapure® AdenoPACK protocols are simple and fast to perform and use the following ste





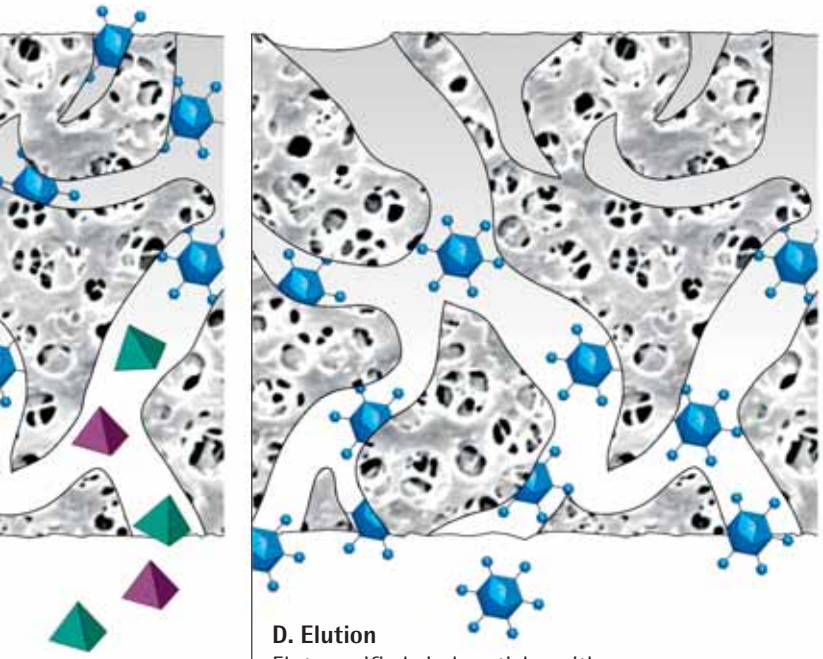
A. Sample Preparation
1. Infect HEK 293 cells with adenovirus stock until most show cytopathic effects. Harvest cells and lyse by freeze|thaw cycles. Remove cell debris by centrifugation.
2. Digest nucleic acids with Benzonase®, filter the Benzonase treated supernatant and dilute with 10x loading buffer.

B. Sample Loading
Pass|centrifuge the prepared supernatant through the Adeno-PACK units. When using Adeno-PACK 100, use a single unit for up to 60 ml virus culture supernatant or use both units in tandem for up to 200 ml.

C. Washing
Wash away residual medium, contaminating proteins and nucleic acids with washing buffer.

Elution 15 min. Total 100 min.

ps to concentrate and purify adenovirus type 5 strains.

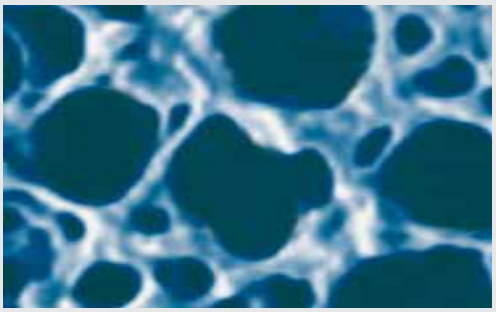


D. Elution
Elute purified viral particles with elution buffer.

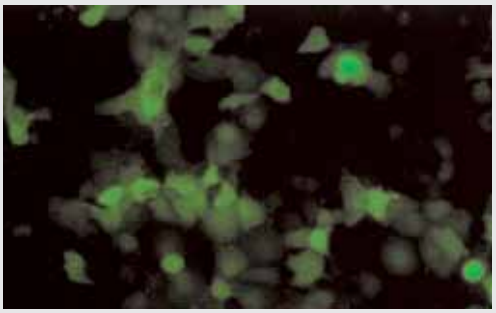
E. Final concentration | buffer exchange
Vivaspin 20 may be used to exchange elution buffer for appropriate physiological or storage buffer but also for virus concentration.

How to use AdenoPACK

AdenoPACK Membrane Adsorbers
The Sartorius Stedim Biotech ion exchange membrane adsorber technology used in AdenoPACK encompasses the advantages of open structures of membranes with the benefits of classical chromatography.



Membrane adsorbers are ideal for virus purification. Large flow through pores allow unrestricted adenovirus access and convective transport speeds up purification.



Photography: kindly provided by Dr. Lux, University Hospital and University of Applied Sciences, Mannheim. COS-1 cells infected with Ad5 GFP-constructs after purification and concentration with Vivapure® AdenoPACK 100. (Fluorescent cell assay picture)

Fluorescent cell assay shows excellent virus infectivity
Recombinant Adenovirus-GFP eluate, purified with AdenoPACK 100 membrane adsorber technology, was used to infect HeLa cells. As the construct contains a GFP gene, successfully transfected cells can be easily identified by fluorescence microscopy. Cell viability and infection rate, determined by microscopy indicate the high purity of the virus concentrates and the low levels of residual endotoxins allow direct use.

Lentivirus Purification with LentiSELECT kits

LentiSELECT 40
The LentiSELECT lentivirus purification and concentration kit offers researchers who need to recover up to 8×10^8 purified recombinant lentivirus particles for in-vitro transfection a fast, safe and easy to use solution. Like the AdenoPACK kits, they include all reagents and devices necessary for purification and concentration of VSV-G pseudotyped lentivirus from HEK293 cell cultures in under one hour. Optimal recoveries are achieved due to an innovative membrane chromatography assembly, making the use of pre-filters for this configuration obsolete.

LentiSELECT advantages

Fast and easy virus purification
- Purification completed in under one hour
- Kit as easy to use as filtration

No need for expensive instruments
- Lentivirus purification with LentiSELECT is independent of equipment such as ultracentrifuges

High virus purity
- Achieve pure virus due to a chromatography purification for your experiments instead of a crude and variable cell culture supernatant pellet

Optimal for multiple virus construct screening
- Four purification runs can be conducted in parallel with one kit

Complete Kit
- Including LentiSELECT units for virus purification, Vivaspins for concentration|buffer exchange and all buffers and syrings necessary

Low endotoxin levels
- High cell viability and infection rates due to endotoxin levels of < 0.025 EU/ml

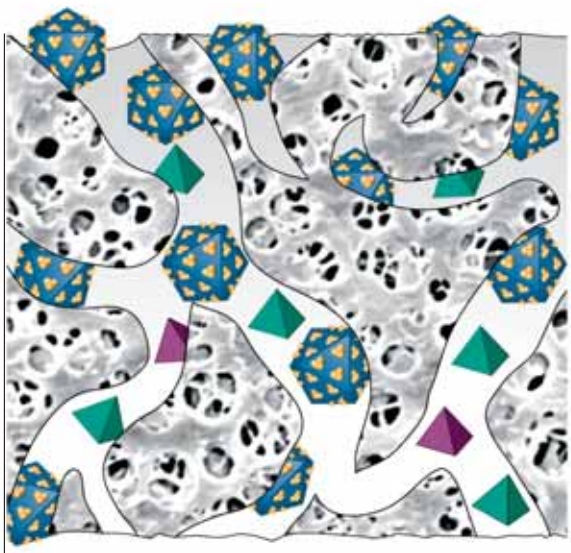
Purification results from preparations with VSV-G pseudotyped lentivirus constructs

Purification method	Process time	Eluate	Recovery***	Viral Particles
LentiSELECT 40|40 ml culture	45 min.	4 ml	28%	8×10^8
Ultracentrifugation|40 ml culture	2.5 hours	0.15 ml	19%	6×10^8

**before buffer exchange

Sample Preparation 5 min. **Sample Loading 20 min.** **Washing 5 min.**

Easy to follow and fast protocol for virus purification. The Vivapure® LentiSELECT protocol is simple and fast to perform and use the following steps



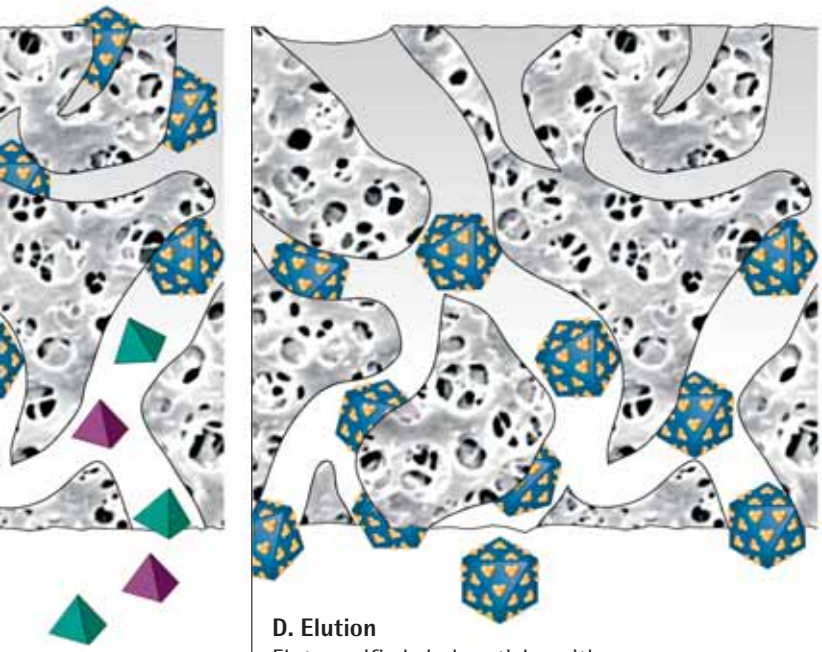
A. Sample Preparation
1.Culture HEK 293 cells and transfect with your cells with packaging plasmid mix and your expression construct.
2. Equilibrate LentiSELECT units by washing with 40 ml 1 × loading buffer

B. Sample Loading
Place end of the feedtube into one 15 cm disch and aspirate the supernatant. Repeat this step with a second dish (do not exceed 40 ml). Pass the sample slowly through the LentiSELECT unit.

C. Washing
Wash away residual medium, contaminating proteins and nucleic acids with washing buffer.

Elution 15 min. Total 45 min.

to concentrate and purify VSV-G pseudotyped lentivirus.

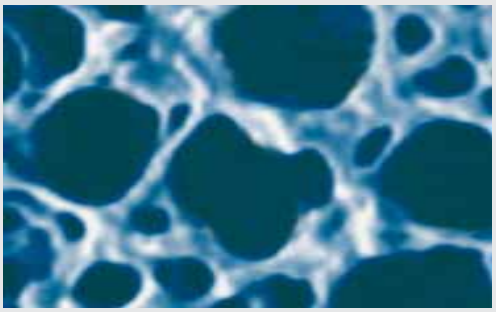


D. Elution
Elute purified viral particles with elution buffer.

E. Final concentration | buffer exchange
Vivaspin 20 may be used to exchange elution buffer for appropriate physiological or storage buffer but also for virus concentration.

Lentivirus purification with LentiSELECT

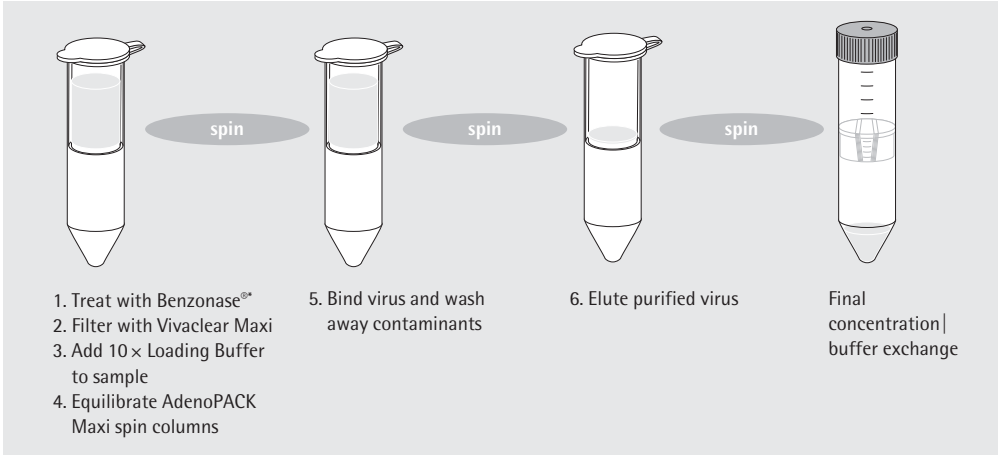
LentiSELECT Membrane Adsorbers
The Sartorius Stedim Biotech ion exchange membrane adsorber technology used in LentiSELECT encompasses the advantages of open structures of membranes with the benefits of classical chromatography.



Membrane adsorbers are ideal for virus purification. Large flow through pores allow unrestricted lentivirus access and convective transport speeds up purification.

Vivapure® AdenoPACK 20 – The optimal kit for construct screening

Vivapure® AdenoPACK 20 is the downscale kit in the AdenoPACK series, purifying up to 1×10^{12} viral particles from 20 ml cell culture. Especially when testing new constructs, parallel and fast purifications of different adenoviruses are essential. This kit allows the rapid, simple and affordable spin column based purification of 6 different samples in parallel and bridges a gap in the CsCl density gradient method – for the first time adenovirus can efficiently be purified from less than 100 ml cell culture volume!



1. Treat with Benzonase®*
2. Filter with Vivaclear Maxi
3. Add 10 × Loading Buffer to sample
4. Equilibrate AdenoPACK Maxi spin columns

5. Bind virus and wash away contaminants

6. Elute purified virus

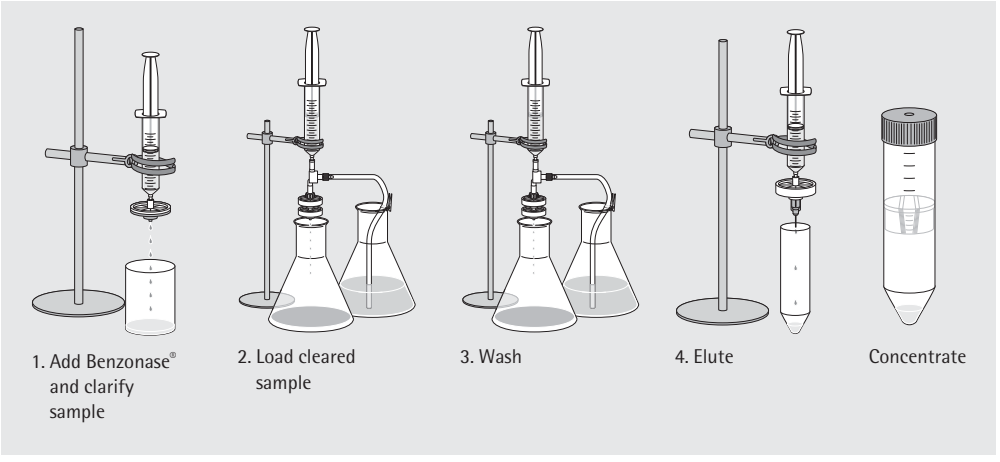
Final concentration | buffer exchange

Typical Performance
For a normal yielding vector, 1 × 15 cm culture plate purified using this method yields up to 1×10^{12} viral particles.

* 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.

Vivapure® AdenoPACK 100 – Fast purification of up to 1×10^{13} viral particles

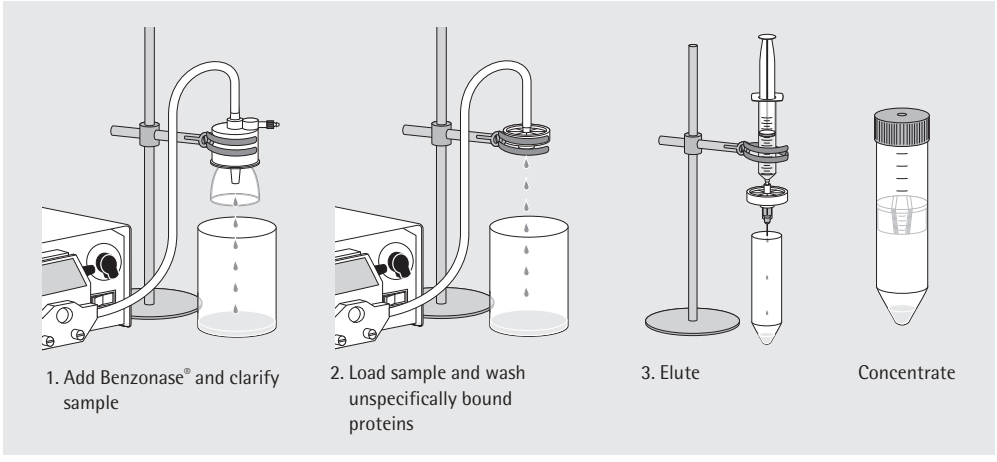
Vivapure® AdenoPACK 100 is optimally suited for adenovirus purification for up to 200 ml cell culture for in vitro transfection. This flexible kit contains two AdenoPACK 100 units, which can be either used in tandem for the purification of up to 200 ml cell culture for recovering 1×10^{13} viral particles or individually for purifying $1–3 \times 10^{12}$ viral particles from up to 60 ml cell culture. The purification is pursued manually with a syringe optimally attached to a retort stand. However, for even more convenience, protocols are provided for optionally running the virus purification with a peristaltic pump or with an infusion pump, in additional to detailed instructions for a manual operation supplied with the Kit. The accessories needed for the operation with a pump are supplied as individual products.



Typical Performance
For a normal yielding vector, 10 × 15 cm culture plate purified using this method yields up to 1×10^{13} viral particles.

Vivapure® AdenoPACK 500 – Pump driven Kit for larger volumes

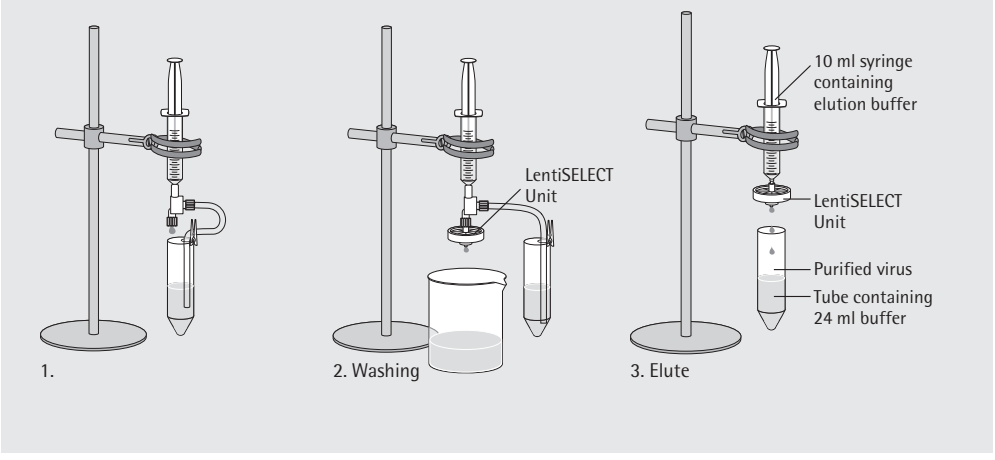
Vivapure® AdenoPACK 500 is the direct upscale kit to the AdenoPACK 100, for adenovirus purification. In only 2 hours up to 3×10^{13} adenovirus particles are purified and concentrated from 500 ml cell culture. This completely ready-to-use kit is conveniently operated by a laboratory pump, offering optimal flow control and minimal hands-on time. This easy to use product replaces lengthy and inefficient cesium chloride density gradient methods.



Typical Performance
For a normal yielding vector, 25 × 15 cm culture plate purified using this method yields up to 3×10^{13} viral particles.

Vivapure® LentiSELECT 40 – Fast purification of up to 8×10^8 viral particles

Vivapure® LentiSELECT 40 is optimally suited for lentivirus purification for up to 40 ml cell culture and contains all components necessary for 4 purifications. Up to 8×10^8 viral particles are recovered in less than one hour. In contrast to traditional ultracentrifugation methods, virus purification with Vivapure® LentiSELECT is fast and simple, without the need for expensive equipment like an ultracentrifuge. Additionally, this chromatographic procedure leads to pure virus samples in contrast to the crude ultracentrifuge pellet, resulting in higher reproducibility and increased gene transfer efficiency.



Typical Performance
For a normal yielding vector, 2×15 cm culture plate purified using this method yield up to 8×10^8 particles.

Technical Data

Vivapure® AdenoPACK 20

Kit Specifications	
Sample size	20 ml of cell culture
Number of purifications	6 × 20 ml
Virus particles (VP) per ml	Typically up to $1 \times 10^{11} - 10^{12}$
VP/IU	50 – 100
Processing time	Typically 1 hour
Endotoxin level	< 0.025 EU/ml

Vivapure® AdenoPACK 100

Kit Specifications	
Sample size	20 – 200 ml of cell culture
Number of purifications	2 × 20 – 60 ml 1 × 200 ml
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

Vivapure® AdenoPACK 500

Kit Specifications	
Sample size	500 ml of cell culture
Number of purifications	1 × 500 ml
Virus particles (VP) per ml	Typically up to 3×10^{13}
VP/IU	20 – 50
Processing time	Typically 2 hours
Endotoxin level	< 0.025 EU/ml

Vivapure® LentiSELECT 40

Kit Specifications	
Sample size	40 ml of cell culture
Number of purifications	4 × 40 ml
Virus particles (VP) per ml	Typically up to 8×10^{8}
VP/IU	5 – 15
Processing time	Typically 45 minutes
Endotoxin level	< 0.025 EU/ml

Ordering Information

Vivapure® AdenoPACK 20 contents and ordering Information	
Vivapure® AdenoPACK 20	VS-AVPQ020
AdenoPACK Maxi 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
Instructions	1 each for kit and Vivaspin

Vivapure® AdenoPACK 100 contents and ordering Information	
Vivapure® AdenoPACK 100	VS-AVPQ101
Vivapure® AdenoPACK 100 RT*	VS-AVPQ102
AdenoPACK 100 units	2
Minisart Plus	4
10 ml syringe	4
Tubing set	2
50 ml syringe	2
Loading buffer (10×)	1 × 25 ml
Washing buffer	1 × 120 ml
Elution buffer	1 × 20 ml
Benzonase® 12.5 U/µl	200 µl
Vivaspin 20, 100 kDa MWCO	4
Instructions	1 each for Kit and Vivaspin

AdenoPACK 100 Accessories	
VS-AVPA001	Pump tubing set for Vivapure® AdenoPACK 100

* AdenoPACK 100 RT does not contain Benzonase®*

Vivapure® AdenoPACK 500 contents and ordering Information	
Vivapure AdenoPACK 500	VS-AVPQ501
AdenoPACK 500 unit	1
Sartopore 2 150	1
Tubing set	2
10 ml syringe	1
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
Instructions	1 each for Kit and Vivaspin

Vivapure® LentiSELECT 40 contents and ordering Information	
Vivapure LentiSELECT 40	VS-LVPQ040
LentiSELECT unit	4
50 ml syringe	4
10 ml syringe	4
Tube set with one-way valve	4
10 × Loading buffer	30 ml
Washing buffer	150 ml
Elution buffer	20 ml
Vivaspin 20, 100 kDa MWCO	8
Operating manual	1 each for Kit and Vivaspin

Sales and Service Contacts

For further contacts, visit www.sartorius-stedim.com

Europe

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen

Phone +49.551.308.0
Fax +49.551.308.3289

www.sartorius-stedim.com

Sartorius Stedim Systems GmbH
Schwarzenberger Weg 73–79
34212 Melsungen

Phone +49.5661.71.3400
Fax +49.5661.71.3702

www.sartorius-stedim.com

France
Sartorius Stedim Biotech S.A.
Z.I. des Paluds
Avenue de Jouques – BP 1051
13781 Aubagne Cedex

Phone +33.442.845600
Fax +33.442.845619

Sartorius Biotechnologie S.A.S.
4, rue Emile Baudot
91127 Palaiseau Cedex

Phone +33.1.6919.2100
Fax +33.1.6920.0922

Austria
Sartorius Stedim Austria GmbH
Franzosengraben 12
A-1030 Vienna

Phone +43.1.7965763.18
Fax +43.1.796576344

Belgium
Sartorius Biotech N.V.
Leuvensesteenweg, 248/B
1800 Vilvoorde

Phone +32.2.756.06.80
Fax +32.2.756.06.81

Denmark
Sartorius Stedim Nordic A/S
Himmelev Bygade 49
4000 Roskilde

Phone +45.70.23.4400
Fax +45.46.30.4030

Italy
Sartorius Stedim Italy S.p.A.
Via dell'Antella, 76/A
50012 Antella-Bagno a Ripoli (FI)

Phone +39.055.63.40.41
Fax +39.055.63.40.526

Netherlands
Sartorius Biotech B.V.
Edisonbaan 24
3439 MN Nieuwegein

Phone +31.30.6025080
Fax +31.30.6025099

Spain
Sartorius, S.A.
C/Isabel Colbrand 10–12,
Planta 4, Oficina 121
Polígono Industrial de Fuencarral
28050 Madrid

Phone +34.91.3586102
Fax +34.91.3588804

Switzerland
Sartorius Stedim Switzerland GmbH
Lerzenstrasse 21
8953 Dietikon

Phone +41.1.746.50.00
Fax +41.1.746.50.50

U.K.
Sartorius Stedim UK Limited
Longmead Business Park
Blenheim Road, Epsom
Surrey KT19 9 QQ

Phone +44.1372.737100
Fax +44.1372.720799

America

USA
Sartorius Stedim North America Inc.
131 Heartland Blvd.
Edgewood, New York 11717

Toll-Free +1.800.368.7178
Fax +1.631.254.4253

Sartorius Stedim SUS Inc.
1910 Mark Court
Concord, CA 94520

Phone +1.925.689.6650
Toll Free +1.800.914.6644
Fax +1.925.689.6988

Sartorius Stedim Systems Inc.
2800 Baglyos Circle
Bethlehem, PA 18020

Phone +1.610.866.4800
Toll-Free +1.800.258.9000
Fax +1.610.866.4890

Asia | Pacific

India
Sartorius Biotech India Private Ltd.
10, 6th Main, 3rd Phase Peenya
KIADB Industrial Area
Bangalore – 560 058

Phone +91.80.2839.1963|0461
Fax +91.80.2839.8262

Japan
Sartorius Stedim Japan K.K.
KY Building, 8–11
Kita Shinagawa 1-chome
Shinagawa-ku
Tokyo 140-0001

Phone +81.3.3740.5407
Fax +81.3.3740.5406

Malaysia
Sartorius Stedim Malaysia Sdn. Bhd.
Lot L3-E-3B, Enterprise 4
Technology Park Malaysia
Bukit Jalil
57000 Kuala Lumpur

Phone +60.3.8996.0622
Fax +60.3.8996.0755

Singapore
Sartorius Stedim Singapore Pte. Ltd.
10, Science Park Road, The Alpha
#02-25, Singapore Science Park 2
Singapore 117684

Phone +65.6872.3966
Fax +65.6778.2494

Australia
Sartorius Stedim Australia Pty. Ltd.
Unit 17/104 Ferntree Gully Road
Waverley Business Park
East Oakleigh, Victoria 3166

Phone +61.3.9590.8800
Fax +61.3.9590.8828

Specifications subject to change without notice. Printed in Germany on paper that has been bleached without any use of chlorine. W/sart-239 · G.
Publication No.: SL-1518-e07104 · Order No.: 85030-530-78