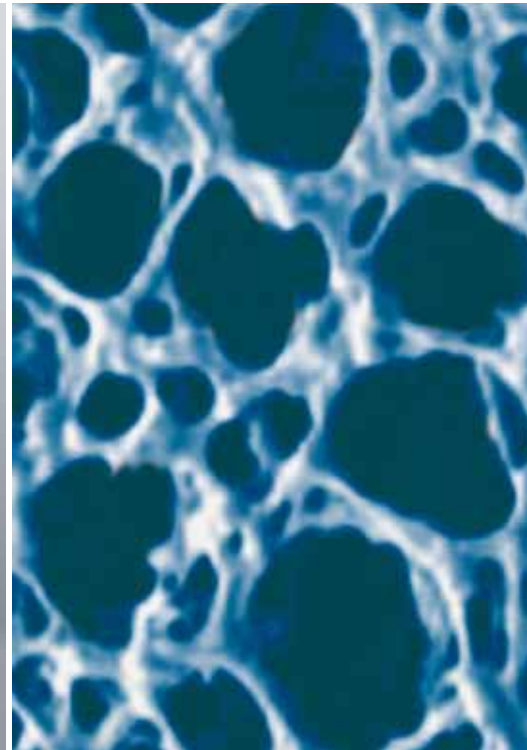




sartorius stedim
biotech

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 sample	1 hour	1 ml	65–70%	$1 \times 10^{11-12}$
AdenoPACK 100 60 ml sample	1–2 hours	1 ml	65%	$1–3 \times 10^{12}$
AdenoPACK 100 200 ml sample	2 hours	1 ml	80%	1×10^{13}
AdenoPACK 500 500 ml sample	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.

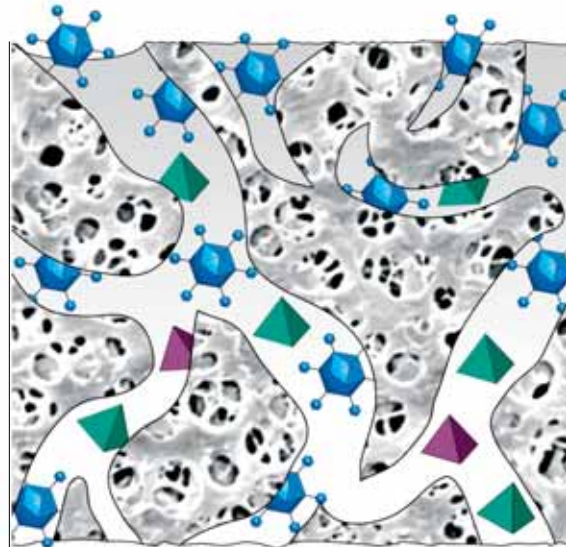


Washing 10 min.

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

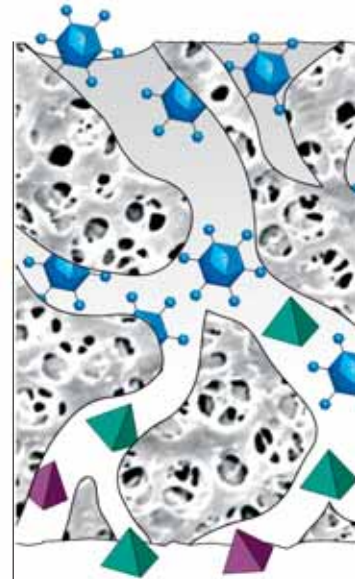
A. Sample Preparation

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.



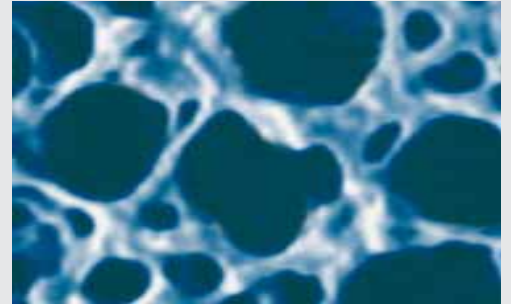
C. Washing

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

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.



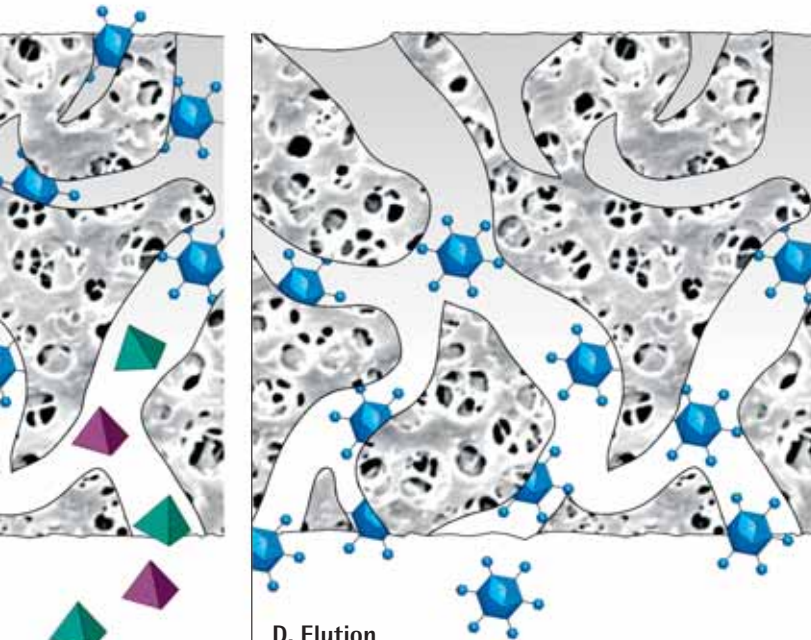
Membrane adsorbers are ideal for virus purification. Large flow through pores allow unrestricted adenovirus access and convective transport speeds up purification.



Elution 15 min.

Total 100 min.

Steps to concentrate and purify adenovirus type 5 strains.

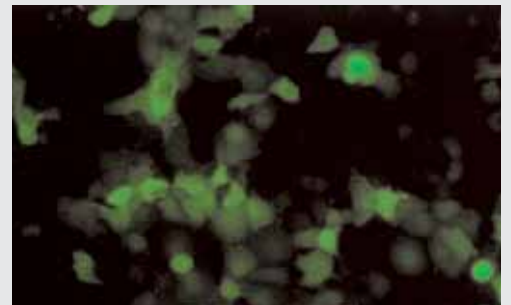


D. Elution

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.



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 | 500 | 1000

The LentiSELECT lentivirus purification and concentration kits offers researchers who need to recover up to $4-5 \times 10^9$ /ml purified recombinant lentivirus particles for in-vitro transfection a fast, safe and easy to use solution. Like the Adeno-PACK kits, they include all reagents and devices necessary for purification and concentration of VSV-G pseudotyped lentivirus from HEK293 cell cultures.

These straight forward kits replace time-consuming ultracentrifugation protocols, which typically take about one day for large sample volumes, thus reducing the purification time to only a few hours.

LentiSELECT kits are offered as LentiSELECT 40, LentiSELECT 500 and LentiSELECT 1000 for the purification and concentration of VSV-G pseudotyped lentivirus from 40 ml to 1000 ml cell culture, leading to $8 \times 10^8 - 1 \times 10^{10}$ purified infective particles. For each sample volume, the most convenient handling method is offered. To this end, 40 ml sample volumes are processed manually with LentiSELECT 40, while LentiSELECT 500 and 1000 are pump driven kits.

LentiSELECT advantages

Fast and easy virus purification

- Purification completed in one to six hours, depending on sample volume
- 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

- With LentiSELECT 40, four purification runs can be conducted in parallel with one kit

Complete Kits

- Including LentiSELECT units for virus purification, Vivaspins for concentration| buffer exchange and all buffers and syringes 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	Infective Viral Particles
LentiSELECT 40 40 ml sample	45 min.	0.2 ml*	50%	8×10^8
LentiSELECT 500 500 ml sample	3 hours	1 ml*	35%	$2-5 \times 10^9$
LentiSELECT 1000 1000 ml sample	6 hours	2 ml*	35%	1×10^{10}
Ultracentrifugation 500 ml sample	10-11 hours	0.5 ml	25%	3×10^9

*After desalting|buffer exchange

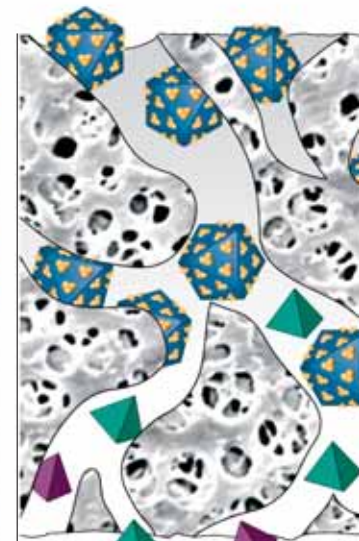
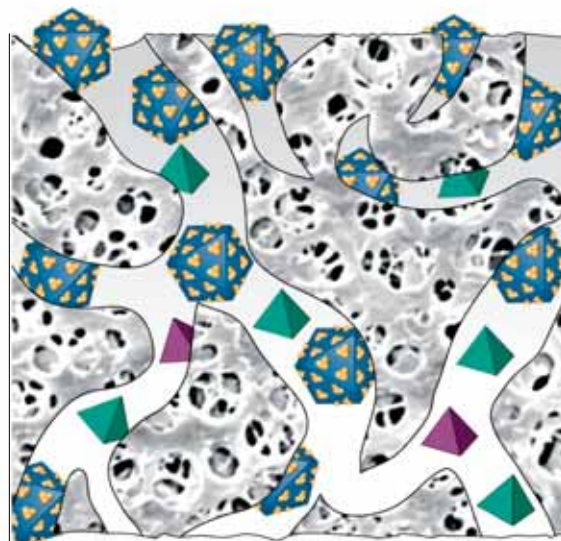


Sample Preparation 5 min.

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 dish and aspirate the supernatant. Repeat this step with a second dish (do not exceed 40 ml). Pass the sample slowly through the LentiSELECT unit.

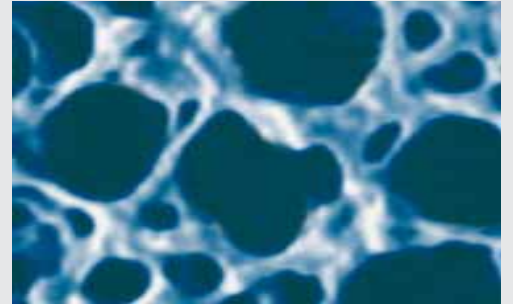
C. Washing

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

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.



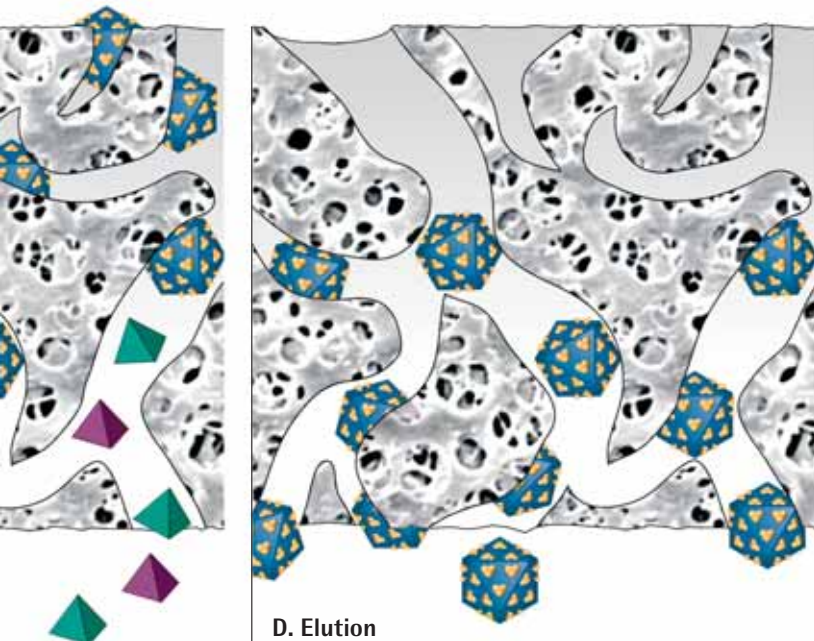
Membrane adsorbers are ideal for virus purification. Large flow through pores allow unrestricted lentivirus access and convective transport speeds up purification.



Elution 15 min.

Total 45 min.*

to concentrate and purify VSV-G pseudotyped lentivirus.



D. Elution

Elute purified viral particles with elution buffer.

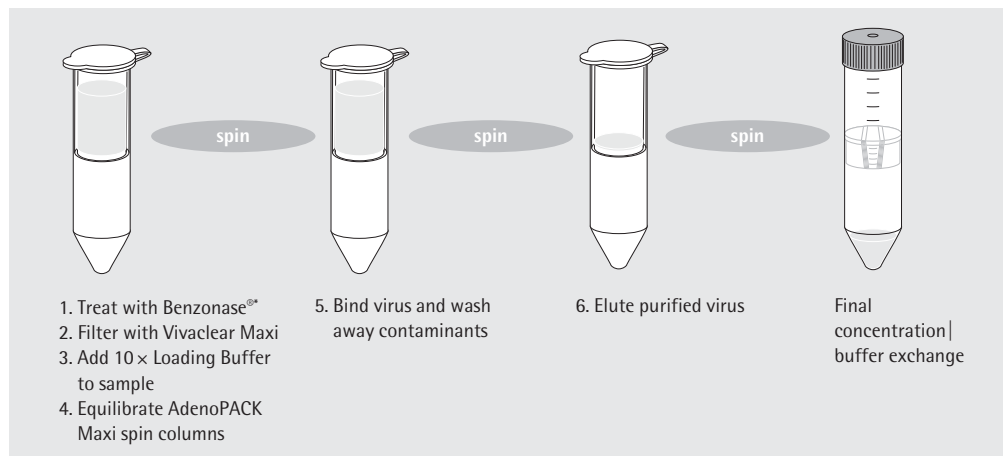
E. Final concentration | buffer exchange

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

* Example for Vivapure LentiSELECT 40

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!



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.

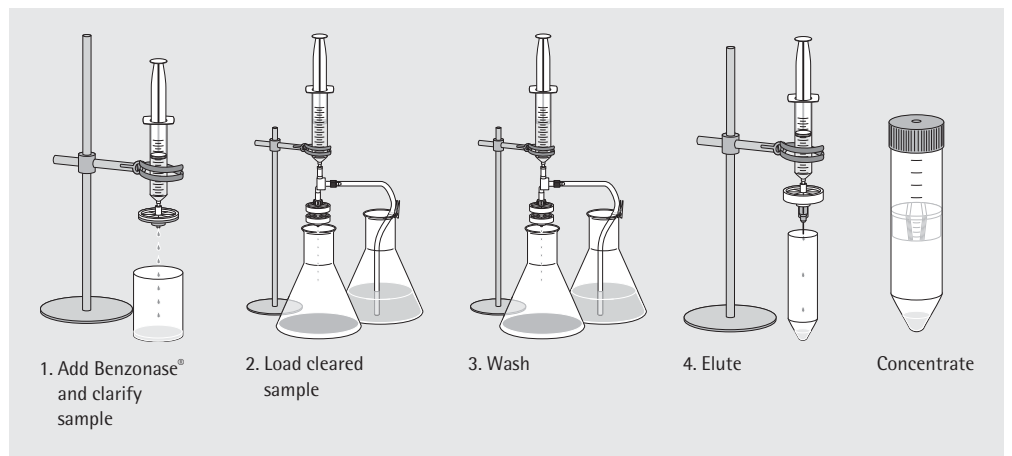
Vivapure® AdenoPACK 100 – Fast purification of up to 1×10^{13} viral particles

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 addition 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 plates purified using this method yield up to 1×10^{13} viral particles.



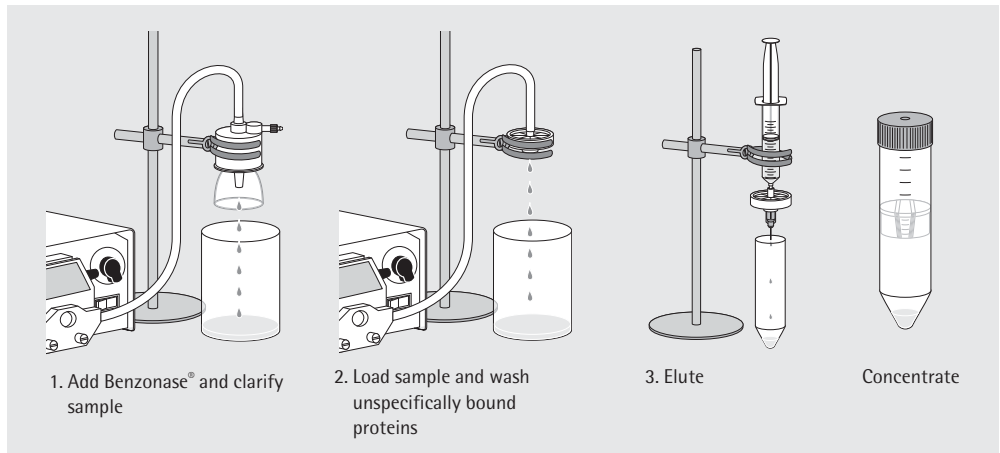
Vivapure® AdenoPACK 500 – Pump driven Kit for larger volumes

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 plates purified using this method yield up to 3×10^{13} viral particles.



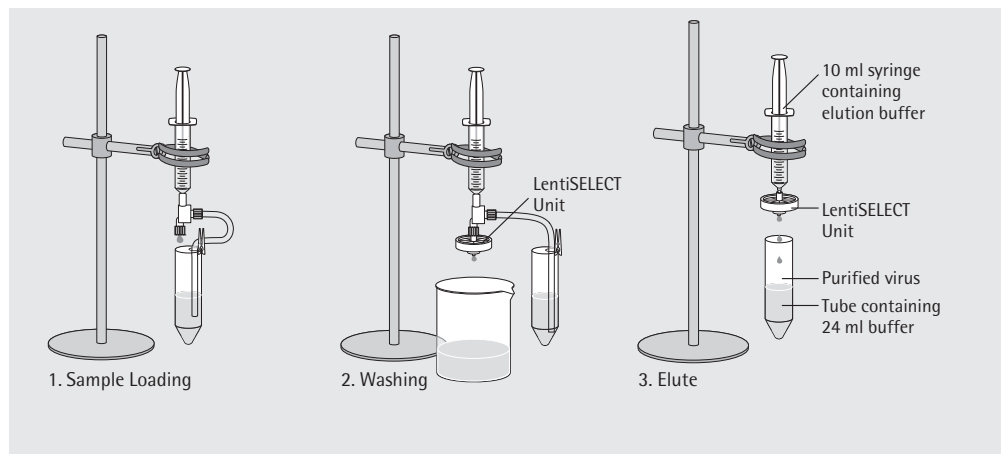
Vivapure® LentiSELECT 40 – Fast purification of up to 8×10^8 viral particles

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 plates purified using this method yield up to 8×10^8 particles.



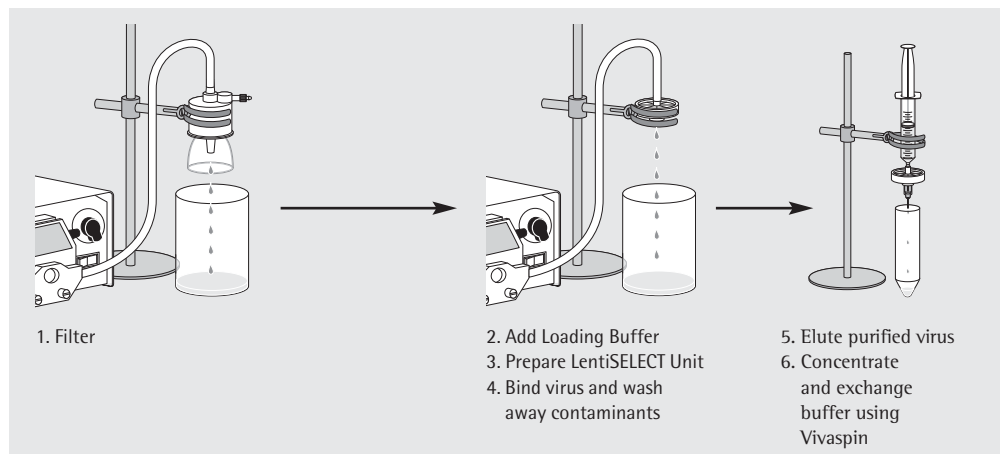
Vivapure® LentiSELECT 500 – Fast purification of up to $2-5 \times 10^9$ infective particles per ml from 500 ml

Vivapure® LentiSELECT 500 is optimally suited for VSV-G pseudotyped lentivirus purification from up to 500 ml cell culture. This kit contains all reagents and devices necessary for purifying up to $2-5 \times 10^9$ infective particles from 500 ml cell culture volume. The whole purification procedure is simply operated by a laboratory pump, which minimizes hands-on time. Unlike conventional purification methods as ultracentrifugation, Vivapure LentiSELECT 500 offers a fast and simple method for purifying VSV-G pseudotyped lentiviruses, which makes expensive purification equipment like ultracentrifuges redundant.



Typical Performance

For a normal yielding vector, 500 ml cell culture purified using this method yield up to $2-5 \times 10^9$ infective units in 1 ml (total volume 1 ml)



Vivapure® LentiSELECT 1000 – Pump driven Kit for larger volumes

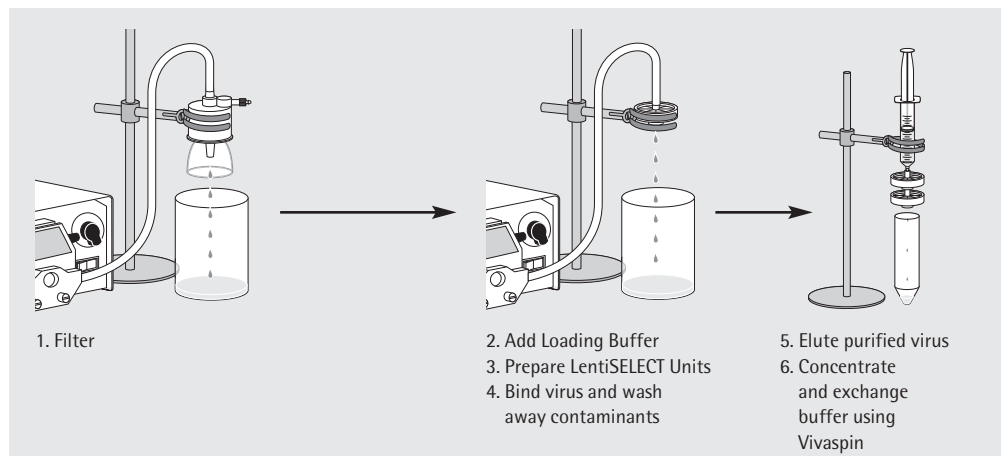
Vivapure® LentiSELECT 1000 is the direct scale up kit to LentiSELECT 500, for VSV-G pseudotyped lentivirus purification. The rapid 6 hour protocol results in a recovery of $4-5 \times 10^9$ infective particles/ml (total volume 2 ml) from 1000 ml cell culture supernatant. This kit is to be operated by a laboratory pump and contains all necessary buffers and ultrafiltration devices for optimal convenience.

The traditional time consuming ultracentrifugation method is replaced by this fast and simple Vivapure LentiSELECT 1000 kit.



Typical Performance

For a normal yielding vector, 1000 ml cell culture purified using this method yield up to $4-5 \times 10^9$ infective particles in 1 ml (total volume 2 ml)



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
Infective particles (IP) per ml	Typically up to 3×10^9 *
VP/IU	5–15
Processing time	Typically 45 minutes
Endotoxin level	< 0.025 EU/ml

* 0.2 ml final elution sample

Vivapure® LentiSELECT 500

Kit Specifications

Sample size	500 ml cell culture
Number of purifications	1 × 500 ml
Infective particles (IP) per ml	Typically up to $2-5 \times 10^9$ *
Processing time	Typically up to 3 hours
Endotoxin level	< 0.025 EU/ml

* 1 ml final elution sample

Vivapure® LentiSELECT 1000

Kit Specifications

Sample size	1000 ml cell culture
Number of purifications	1 × 1000 ml
Infective particles (IP) per ml	Typically up to $4-5 \times 10^9$ *
Processing time	Typically up to 6 hours
Endotoxin level	< 0.025 EU/ml

* 2 ml final elution sample

Ordering Information

Vivapure® AdenoPACK 20 contents and ordering Information

Vivapure® AdenoPACK 20	VS-AVPQ020
Vivapure® AdenoPACK 20 RT*	VS-AVPQ022
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
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Vivapure® AdenoPACK 500 contents and ordering Information

Vivapure® AdenoPACK 500	VS-AVPQ501
Vivapure® AdenoPACK 500 RT*	VS-AVPQ502
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

* AdenoPACK 20 | 100 | 500 RT does not contain Benzonase®

Vivapure® LentiSELECT 40 contents and ordering Information

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

Vivapure® LentiSELECT 500 contents and ordering Information

Vivapure® LentiSELECT 500	VS-LVPQ500
LentiSELECT unit	1
Sartopore 2 150	1
50 ml syringe	1
Tube set with one-way valve	1
Loading buffer (10 ×)	30 ml
Washing buffer	170 ml
Elution buffer	30 ml
Vivaspin 20, 300 kDa MWCO	2
Operating manual	1 each for Kit and Vivaspin

Vivapure® LentiSELECT 1000 contents and ordering Information

Vivapure® LentiSELECT 1000	VS-LVPQ1000
LentiSELECT units	2
Sartopore 2 150	1
50 ml syringe	1
Tube set with one-way valve	1
Loading buffer (10 ×)	30 ml
Washing buffer	170 ml
Elution buffer	60 ml
Vivaspin 20, 300 kDa MWCO	2
Operating manual	1 each for Kit and Vivaspin

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