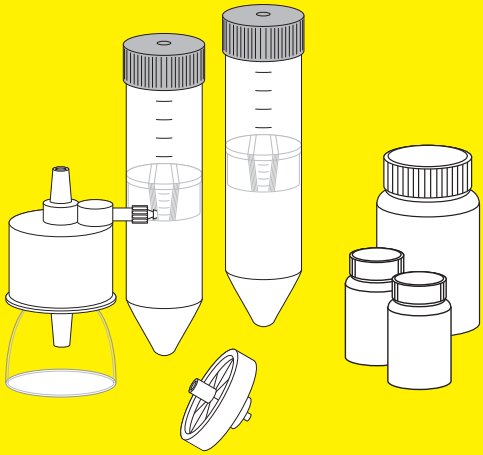
Instructions for Use

Vivapure® Lentiselect 500

VSV-G pseudotyped Lentivirus purification and concentration kit for up to 500 ml cell culture volume (E.g. 25 × 15 cm plates) | For *in vitro* use only



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Vivapure Lentiselect 500 – Introduction

Storage conditions | shelf life
The Lentiselect kit should be stored at room temperature. This kit should be used within 24 months of purchase.

Introduction
This protocol describes the purification of VSV-G pseudotyped Lentivirus with Sartobind Q 100 syringe filters containing a membrane adsorber that selectively binds lentiviral particles. Once bound, virus particles can be further purified by washing away nonspecifically bound proteins before elution and concentration within 3 hours.

The concentrated virus is suitable for *in vitro* and animal studies after buffer exchange into a physiological buffer.

In contrast, traditional ultracentrifugation is a time consuming method, typically taking up to 10 hours for 500 ml. Ready to use filter devices, Sartobind Q 100 units, centrifugal Vivaspin concentrators and buffers make the following purification procedure as easy as filtration.

Vivapure Lentiselect 500

Cat. Number	VS-LVPQ500
Sartobind Q 100 unit	1
Sartopore 2 150 clarifying filter	1
50 ml syringe	1
Tubing set	1
Loading Buffer (10×)	30 ml
Washing Buffer (1×)	170 ml
Elution Buffer (1×)	30 ml
Vivaspin 20, 100 kDa MWCO	2
Technical data sheet	1 each for Kit and Vivaspin

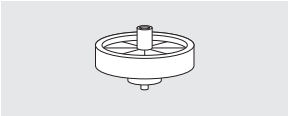
Materials of construction

Sartobind Q 100 MA housing	Polysulfone
Sartopore 2 150 clarifying filter housing	Polypropylene
Sartobind Q membrane	Stabilised RC
Buffer containers	LDPE
Purification buffers	Proprietary

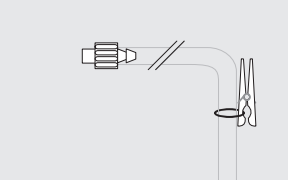
Kit specifications

Sample size	Up to 500 ml of Lentivirus supernatants
Virus particles (VP)	Typically up to $2-5 \times 10^9$
Processing time	Typically 3 hours

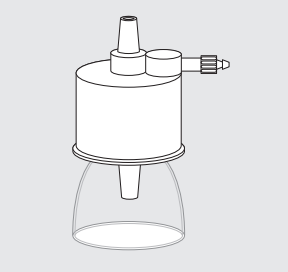
Kit contents



Sartobind Q 100 unit
with protective end caps



1× Pump tube set

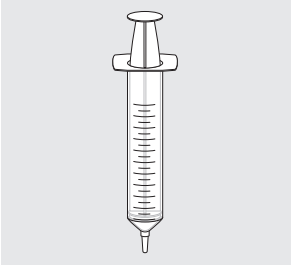


1× Sartopore 2
150 clarifying filter

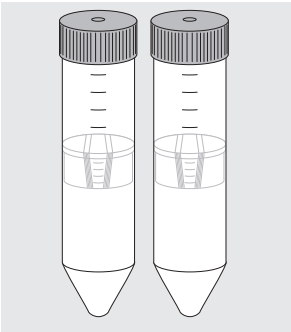
30 ml Loading Buffer (10×)

170 ml Washing Buffer (1×)

30 ml Elution Buffer



50 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
Sterile plastic container for sample handling Sterile 50 ml tube for collection of purified virus
HBSS buffer: Hanks Balanced Salt Solution (1×) [6,3 mM KCl, 0,4 mM KH_2PO_4, 4,2 mM $NaHCO_3$, 138 mM NaCl, 0,3 mM Na_2HPO_4, 5,6 mM D-Glucose] or other buffer of choice

Purification protocol – Overview

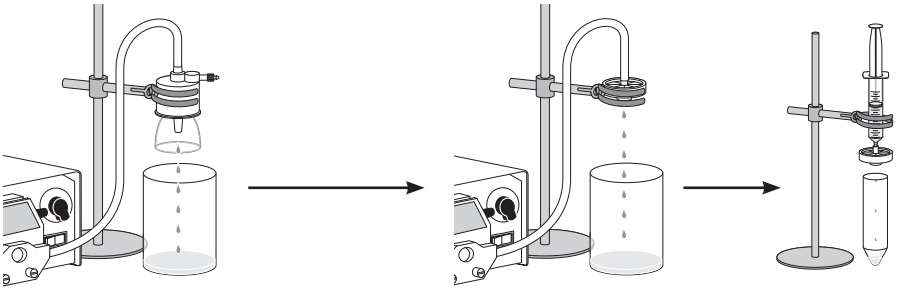
HEK 293 Cells

Grow cells overnight at 10% CO_2,
the cells should not exceed confluency
of 60% for transfection

Transfect HEK 293 cells

1. Incubate cells at 3% CO_2 for 16h
2. Remove medium and add fresh medium
3. Culture cells for further 24 h at 10% CO_2

supernatant, containing lentivirus



1. Filter

2. Add Loading Buffer
3. Prepare Sartobind Q 100 Unit
4. Bind virus and wash away contaminants

5. Elute purified virus
6. Concentrate and exchange buffer using Vivaspin

Purification protocol – Overview

General protocol
The protocol uses the following steps to concentrate and purify VSV-G pseudotyped Lentivirus strains.

Note: This kit contains sufficient materials to concentrate and purify virus from 500 ml virus containing culture medium. The detailed protocols are written as though for a 500 ml preparation, please adjust reagent volumes accordingly for smaller samples.

Virus culture
Transfect HEK 293 cells with Lentivirus plasmid and packaging plasmids. Change medium after 16 hours and incubate for further 24 hours.

Sample preparation
Filter the 500 ml supernatant with the supplied filter unit.

Sartobind Q 100 preparation
Equilibrate the membrane and remove air bubbles from the Sartobind Q 100 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 100 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 100 unit with a syringe. Incubation of the Sartobind Q 100 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
Elution buffer must be exchanged to a buffer of choice (physiological buffer, e. g HBSS) using the Vivaspin 20 concentrators supplied with this kit. Virus titre can be increased through longer centrifugation times than advised in the manual. Please take care not to fall below 500µl – 1 ml volume, as this will lead to aggregation of the virus.

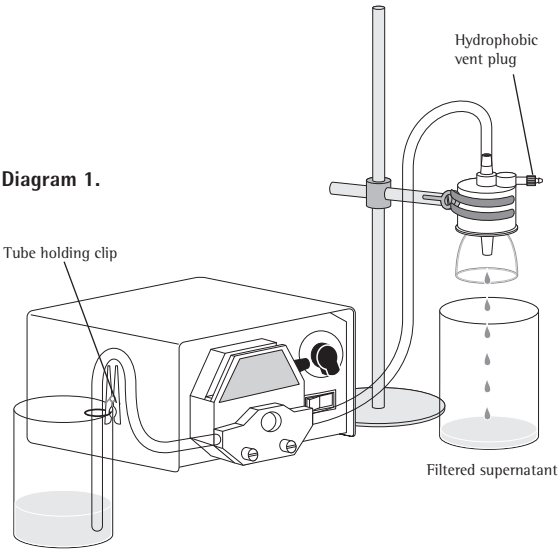
Purification protocol – Techniques

A.) Virus culture
Note: The 10 × Loading Buffer included in the Vivapure Lentiselect 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 and incubate overnight at 37 °C and 10% CO_2. On the next day the cell monolayer should not exceed confluency of 60% for transfection.
Add transfection reagent and respective plasmids, incubate overnight for 16 hours at 37 °C and 3% CO_2. Remove the medium and add 20 ml fresh medium. Culture transfected cells at 37 °C with 10% CO_2 for further 24 hours.

Sample preparation
1. Assemble equipment as shown in diagram 1. Loosen the hydrophobic vent plug in the Sartopore 2 casing. Pump 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.

Diagram 1.



B.) Sartobind Q 100 preparation
Note: Air trapped in the Sartobind Q 100 will reduce viral titre. All the air must be removed from the Sartobind Q 100 unit so that virus particles can bind to the membrane.

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

3. Add 10 × Loading Buffer to destilled water. Volume of final 1 × Loading Buffer | 9 = volume of 10 × Loading Buffer to be added E.g. for 80 ml final diluted volume add 71 ml destilled water to 9 ml 10 × Loading Buffer.

4. Pump 1 × Loading Buffer through the Sartobind Q 100 unit to ensure it is fully wetted.

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

6. Pump through 70 – 80 ml 1 × Loading Buffer 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.

Purification protocol – Techniques

Sample loading | washing

7. Remove the feed tube from the beaker containing 1 × Loading Buffer and place into the container of filtered and pooled virus containing supernatant. Pump Loading Buffer through the Sartobind Q 100 unit at the set speed of 10 ml/min. Collect flow through and treat as biohazard waste.

8. When the supernatant container is almost empty, pour 80 ml 1 × Washing Buffer into the sample container.

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

Diagram 2.

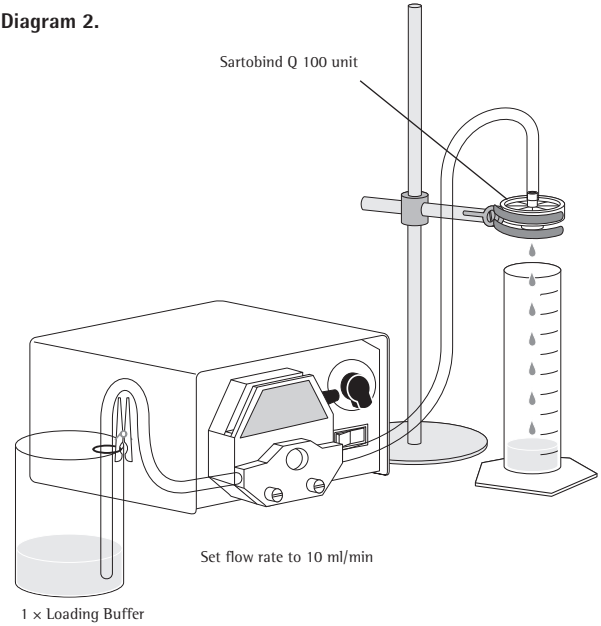
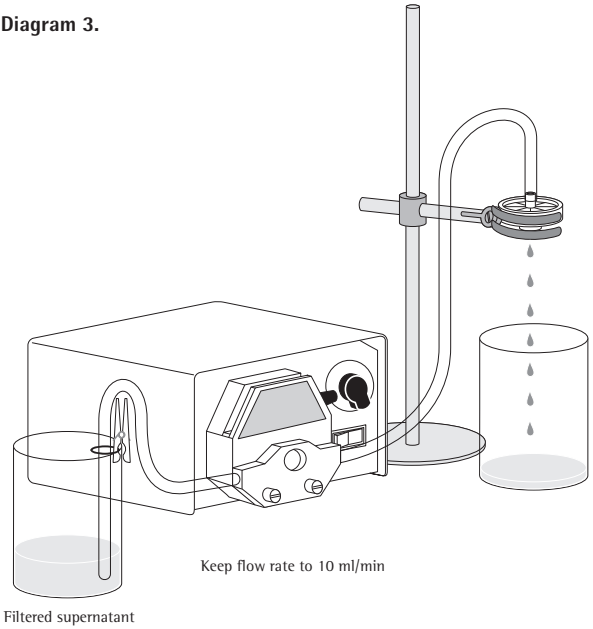


Diagram 3.



Purification protocol – Techniques

Elution
Note: Viral particles are eluted using a buffered solution containing a high level of sodium chloride; to maintain viral infectivity, it is necessary to exchange the purified virus into suitable storage buffer immediately after elution.

10. Fill a fresh 50 ml syringe with 20 ml Elution Buffer and set aside.

11. Detach the Sartobind Q 100 unit from the tubing assembly and attach the filled 50 ml syringe to the inlet.

12. Hold the syringe vertically. Very slowly (drop-by-drop) pass 2,5 ml Elution Buffer through the Sartobind Q 100 unit and collect in a sterile 50 ml tube. (See diagram 4.)

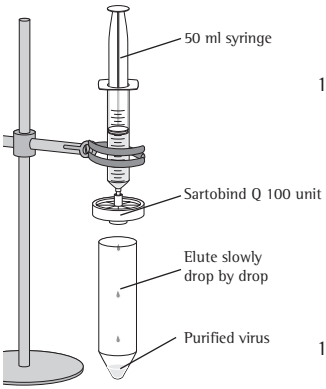
Caution: Press syringe plunger very gently, eluting too quickly will reduce the recovery of purified virus. The optimal flow rate for elution is 1 ml/min; you will achieve this if you can count the individual drops.

13. Leave the syringe (with the remaining 17,5 ml Elution Buffer in it) attached to the Sartobind Q 100 unit and incubate for 5 min at room temperature.

14. Pass the remaining Elution Buffer through the LentiSELCT 500 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 10 – 12.

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

Diagram 4.



Final concentration | buffer exchange
Note: Buffer exchange from elution buffer to buffer of choice must be performed immediately after elution. Virus titre can be increased through longer centrifugation times than advised in the manual. Please take care not to fall below 500µl - 1 ml volume, as this will lead to aggregation of the virus. 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 longterm 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.

16. Divide the eluate and pipette into two Vivaspin 20 centrifugal concentrator

17. Centrifuge for 30 – 40 min at up to 3,000 xg in a swing-out rotor, or in a 25° fixed-angle rotor, with cavities accepting 50 ml conical bottom tubes. The printed graduations should face away from the center of the rotor in case of using fixed angle rotors.

18. 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 500 µl – 1 ml in order to avoid virus aggregation and loss of infectivity.

19. Discard filtrate when sample volume reaches 2 – 3 ml and pool the retentates in one of the Vivaspin 20 and add 10 ml of a buffer of choice (e. g. HBSS). Fill up the other Vivaspin 20 with water or PBS to counterbalance the rotor.

20. Centrifuge for 60 min at up to 3000 × g in a swing-out rotor or in a 25° fixed angle rotor. For concentrating to a lower volume of choice, further centrifugation may be necessary. Do not reduce to a volume of less than 500 µl – 1 ml.

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

22. 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 with a starting titer of $2-5 \times 10^7$ infective particles/ml should yield a range of up to $2-5 \times 10^9$ infective particles/ml (total volume 1 ml).

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.

(Optional) Sterilization
Some applications require the virus sample to be sterilized. This can be achieved using a Minisart RC 15 0.2 µm (order no. 17761 ACK) that is not included with this kit.

1. Draw concentrated virus sample into a syringe that has a luer lock connector
2. Remove a Minisart from the box
3. Connect the syringe to the luer inlet of the Minisart filter unit
4. Filter the concentrated virus sample in to a tube
5. The filter-sterilized virus will now be ready for use
6. Upon completion of filtration, discard the Minisart

Trouble shooting

Problem	Cause	Answer
Air in the feed tube	Liquid level low in sample container	Do not expel through the Sartobind Q 100 units. Remove the Sartobind Q 100 unit temporarily from the syringe and expel the air. Re-fill the syringe and tube set with liquid, then re-fit Sartobind Q 100 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 100 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 Lentiselect protocol precisely
	Low viral titre in culture	Optimise virus production
	Buffer left in the Sartobind Q 100 unit	After elution, blow air through the Sartobind Q 100 unit to recover all the buffer
Low virus recovery	Virus producing cultures allowed to grow too long may result in decreasing titres	
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
Sartopore 2 150 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-LVPQ500	Vivapure Lentiselect 500, 500 ml culture volume	1
VS-LVPQ1000	Vivapure Lentiselect 1000, 1000 ml culture volume	1
Sartorius products in this kit		
VS2041	Vivaspin 20, 100,000 MWCO PES	2
5441307H4—oo	Sartopore 2 150 0.45–0.2 µm PES	1
93IEXQ42BC-12	Sartobind Q 100	1
Lentiselect 500 Accessories		
VFP001	Masterflex economy drive variable speed peristaltic pump (240 V)	
VFP002	Masterflex economy drive variable speed peristaltic pump (115V)	
VFA012	Masterflex easy load pump head-size 16	

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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