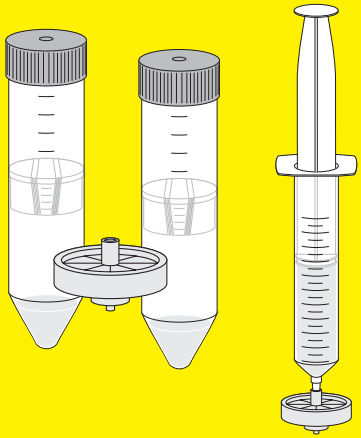
Instructions for Use

Vivapure® Lentiselect 40

Lentivirus (VSV-G pseudotype) purification and concentration kit for preparations up to 40 ml cell culture volume each (E.g. 1–2 × 15 cm plates) | For in vitro use only



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SARTORIUS

Introduction

Storage conditions | shelf life
Kit components should be stored at room temperature.

The kit should be used within 24 months.

Safety advice
Warning: The virus purified using this kit is capable of infecting human or animal cells and could, depending on the gene insert, expose the user to potentially hazardous biological material. Lentiviruses have been designated as Level 2 biological agents. All protocols detailed in these operating instructions must be performed under at least Biosafety Level 2 working conditions.

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

This kit is NOT intended for human or animal diagnostic or therapeutic applications.

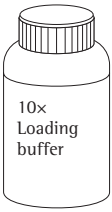
Key features:
- Membrane based purification that selectively binds lentiviral particles.
- Easy to follow protocol.
- Purification in less than 1 hours.
- Scaleable purification technology.

Usage tips:
Take care that air is not trapped in the membrane adsorber or clarifying filter units, as this will reduce the binding of virus.

To ensure optimum performance, the sample can be well clarified before loading.

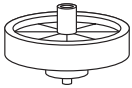
Vivapure Lentiselect 40

Order Number	VS-LVPQ040
Sartobind Q 15 unit	4
50 ml syringe	4
10 ml syringe	4
Tube set with one-way valve	4
10x Loading buffer	30 ml
Washing buffer	170 ml
Elution buffer	30 ml
Vivaspin 20 100 kDa MWCO	8
Operating manual	1 each for Kit and Vivaspin



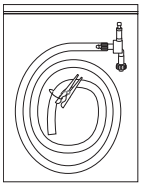
10× Loading buffer

Elution buffer

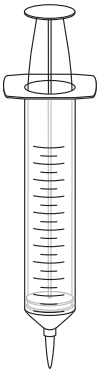


Sartobind Q 15 membrane adsorber

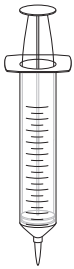
Washing buffer



Tube set with one-way valve



50 ml syringes



10 ml syringes

Vivaspin 20
100 kDa MWCO

Additional material required but not supplied

Centrifuge with fixed angle rotor accepting 50 ml falcon tubes

Retort stand and clamp

Sterile 50 ml tubes or plastic containers for sample handling

100 ml plastic beaker for collecting loading and washing waste

5 ml of HBSS [Hanks' Balanced Salt Solution (1×): 5.3 mM Kcl, 0.4 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 133 mM NaCl, 0.3 mM Na_2HPO_4, 5.6 mM D-Glucose]
Eppendorf Tubes

Purification protocol – Techniques

A) Virus culture
For each preparation, grow HEK 293 cells in up to 40 ml total culture medium (E.g. 2x 15 cm plates), to 50–70 % confluency.

Replace the cell culture medium with new growth medium and transfect cells with transfection reagent including a packaging | expression plasmid according to the manufacturer's instructions.

Depending on your transfection protocol we recommend you incubate cells overnight, then change the media and replace it with fresh, complete culture medium.

B) Equipment preparation
Dilute 5 ml 10× Loading buffer with 45 ml sterile ultrapure water in a sterile 50 ml falcon tube. Attach the tube set to the 50 ml syringe as shown in the diagram and clamp this to a retort stand. Place the feed tube into the 1× Loading buffer and draw some up into the syringe (a). Push this liquid, and the air in the syringe, out through the one-way valve back into the container (b). Repeat until all the air is removed from the syringe. (See figure 1.)

Fill the syringe with 40 ml 1× Loading buffer.

Attach a Sartobind Q 15 unit to the vent (see figure 2) and press 30–35 ml of Loading buffer through the Sartobind Q 15 unit. Leave about 1–2 ml 1× Loading buffer remaining in the syringe to be sure that air is not introduced to the filters.

C) Sample preparation
Note: It is important to hold the assembly vertical and steady throughout sample loading. This is easier if the filled syringe assembly is clamped to a retort stand before loading.

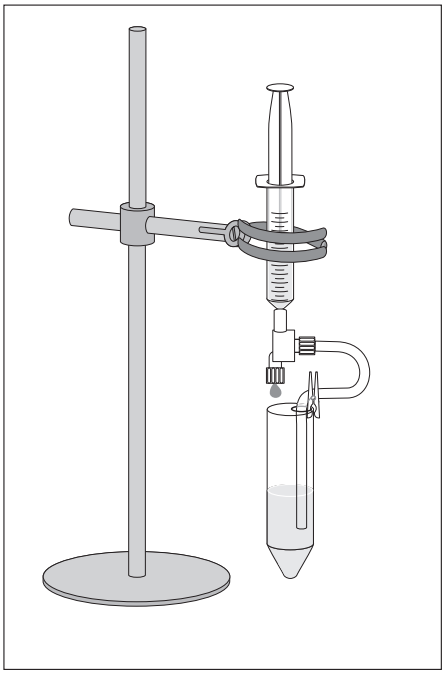


Figure 1

Loading
36–48 (max 72) hours post transfection, place the end of the feed tube into one 15 cm dish and aspirate the supernatant. Continue until the minimum of sample is left in dish but the feed tube remains full. Caution: Make sure that no air enters the unit. Repeat this step with a second dish, the total culture supernatant should not exceed 40 ml. Hold the syringe vertically to ensure even loading of supernatant.

Place the end of the feed tube into the tube with 1× Loading buffer and draw some up until the tube is filled with Loading buffer and the virus containing supernatant is completely drawn into the syringe.

Pass prepared sample solution slowly through the unit. The optimal flow rate for loading is 20 ml/min; you will achieve this if you can count the individual drops. Leave 1-2 ml liquid in the syringe at the end to prevent air entering the unit.

Caution: Press syringe plunger gently. Loading too quickly will reduce the capture of virus particles.

D) Washing

Note: To ensure an efficient changeover from loading to washing, draw up sufficient Washing buffer to just fill the feed tube, then push out through the units to flush the remaining sample solution through before continuing with the main wash.

Place the end of the feed tube into the container with Washing buffer and draw 30 ml up. Caution: Do not draw air into the feed tube. If this happens, see Troubleshooting.

Pass the Washing buffer through the unit. The flow rate for washing may be higher than for loading. Caution: Do not push air through the units during washing.

Leave 1-2 ml liquid in the syringe at the end to prevent air entering the units and continue to elution step.

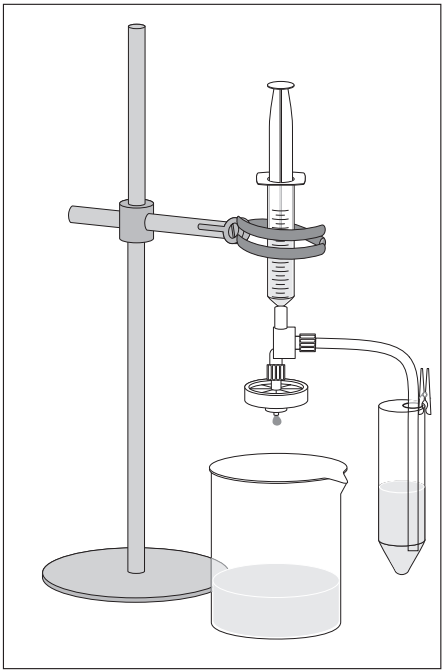


Figure 2

E) 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.

Take a 10 ml syringe and fill with 4 ml Elution buffer and remove any air bubbles.

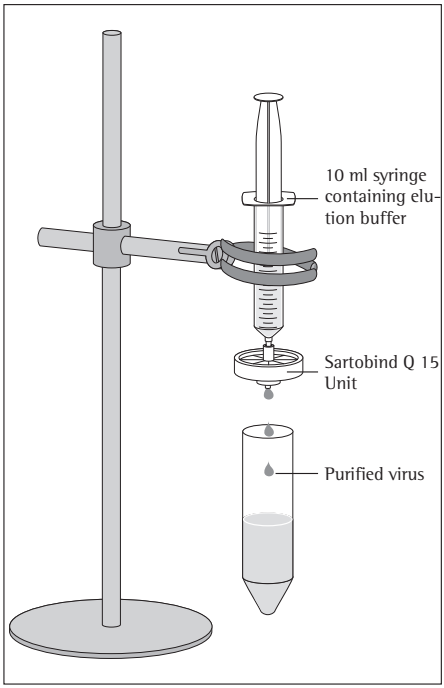
Detach the Sartobind Q 15 unit from the 50 ml syringe and attach to the filled 10 ml syringe.

Hold the syringe vertically. Taking 1–2 minutes, slowly pass 4 ml Elution buffer through the Sartobind Q 15 unit while gently moving the syringe plunger up and down to remove any trapped air in the unit.

Collect the eluate in the tube or directly in the supplied Vivaspin 20.

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

Cap the tube and invert a few times to mix.



F) Final concentration
Note: Further concentrate the viral eluate to increase titer.

Transfer 4 ml of the eluate to a Vivaspin 20 unit. Place the Vivaspin 20 into a centrifuge and counterbalance the rotor with a second concentrator falcon tube filled with equivalent volume of PBS or water. In fixed angle rotors the printed graduations should face away from the centre of the rotor.
Centrifuge for 10 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.

Discard filtrate, then add 3.5 ml HBSS [or other common buffer] to the viral concentrate. Centrifuge at 3,000 × g for 20 min.

Check the volume of the viral concentrate remaining in the upper chamber and if necessary centrifuge again.

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

Avoid bubbles.
Determine viral titre. Aliquot accordingly and store virus at -80°C.

G) Optional: Buffer exchange
Note: It is sometimes necessary that virus is exchanged into physiological buffer before use in tissue culture or cell based assays or into generic storage buffers 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.

Discard filtrate when sample volume reaches 1 ml, and then add storage | physiological buffer to the concentrate to bring the volume up to 10 ml.

Centrifuge again as before and if necessary repeat buffer exchange a second time.

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

Determine viral titre. Aliquot accordingly and store virus at –80°C.

Typical performance:
Depending on the content of your original sample, expect 8×10^8 viral particles in 4 ml eluate. You can achieve higher titres by concentration in the supplied Vivaspins.

(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 15 units. Remove the Sartobind Q 15 unit temporarily from the syringe and expel the air. Re-fill the syringe and tube set with liquid, then re-fit Sartobind Q 15 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 15 unit	Avoid trapping air in the Sartobind Q 15 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 15 unit	After elution, blow air through the Sartobind Q 15 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
Q15X	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

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Sperry Way, Stonehouse Park
GL10 3UT Stonehouse, Gloucestershire, UK

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