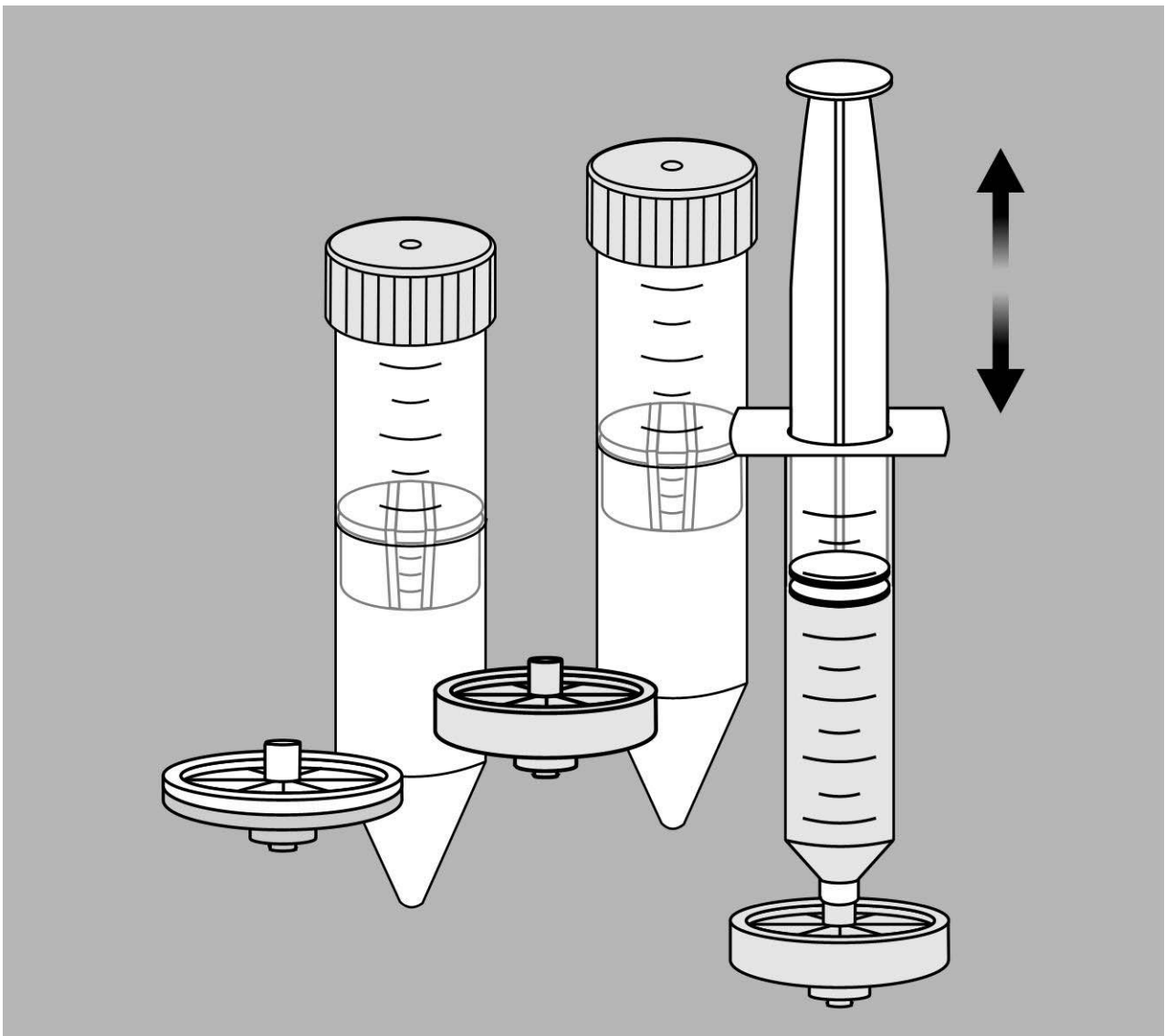


Technical data and operating instructions.
For in vitro use only.

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 x 15 cm plates)



VS-PK-1007-07

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.

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 2 hours.
- Scalable 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 must be well clarified before loading.

Vivapure LentiSelect 40

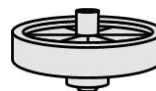
Prototype Number	VS-PK-1007-07
LentiSelect membrane adsorber unit	2
Minisart Hi-flow 0.45 µm PES	2
50 ml syringe	2
10 ml syringe	2
Tube set with one-way valve	2
10x Loading buffer	15 ml
Washing buffer	40 ml
Elution buffer	10 ml
Vivaspin 20 100 kDa MWCO	4
Operating manual	1



10x Loading buffer



Elution buffer



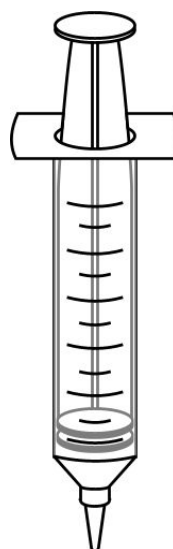
LentiSelect membrane adsorber



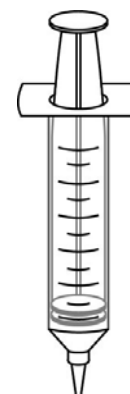
Minisart in blister packs (Yellow)



Washing buffer



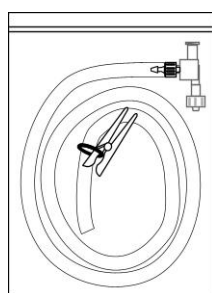
50 ml syringes



10 ml syringes



Vivaspin 20 100 kDa MWCO



Tube set with one-way valve

Additional material required but not supplied

Centrifuge with swing out rotor accepting 50 ml falcon tubes.

Retort stand and clamp.

50 ml of PBS pH 7.4

Sterile 50 ml tubes or plastic containers for sample handling.

100 ml plastic beaker for collecting loading and washing waste.

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 x 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 x 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 x Loading buffer.

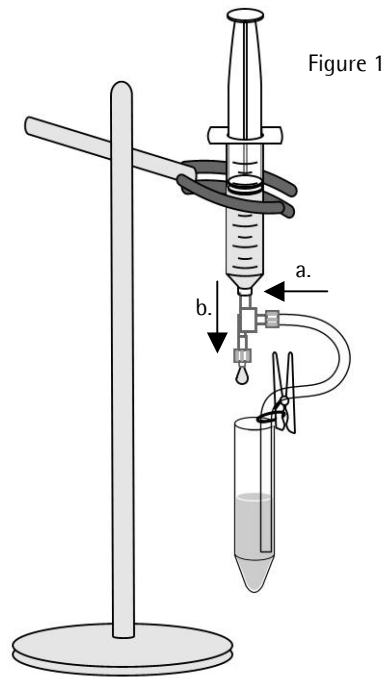
Remove a Minisart from its blister pack and attach it to the vent, finally, attach a LentiSelect unit to the Minisart. (See figure 2).

Press 30 – 35 ml of Loading buffer through the assembled filter units. Leave about 1-2 ml 1 x Loading buffer remaining in the syringe to be sure that air is not introduced to the filters.

Caution: Once wetted, do not push air through the Minisart and LentiSelect unit during filtration as this may block the filter.

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.



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 units. 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.

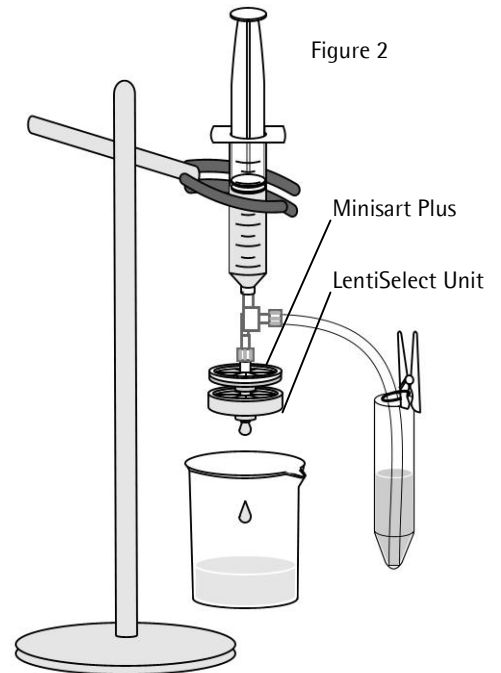
Place the end of the feed tube into the tube with 1 x Loading buffer and draw some up until the tube is filled with Loading buffer and the virus containing supernatant is completely drawn into the syringe.

Pass prepared sample solution slowly through the units. The optimal flow rate for loading is 10 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 units. 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.

Purification protocol – Techniques

E). Elution

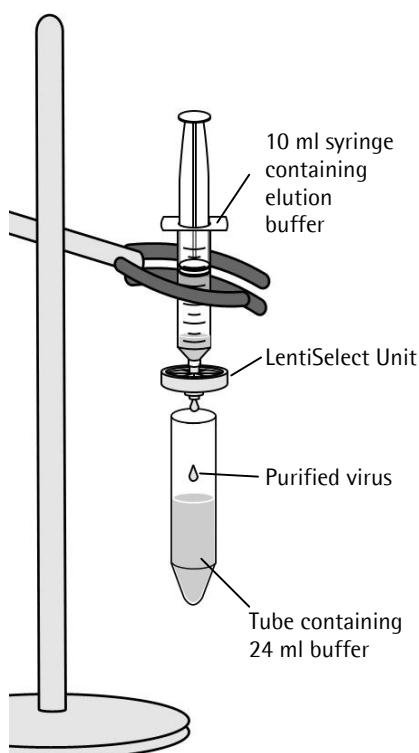
Note: Viral particles are eluted using a buffered solution containing a high level of sodium chloride; to maintain viral infectivity, it is necessary to exchange the purified virus into suitable storage buffer immediately after elution.

Add 24 ml buffer of your choice or 1 x Loading buffer to a 50 ml tube. Take a 10 ml syringe and fill with 4 ml Elution buffer and remove any air bubbles.

Detach the LentiSelect unit from the Minisart (discard Minisart and 50 ml syringe with tube set) and attach to the filled 10 ml syringe.

Hold the syringe vertically. Taking 1 - 2 minutes, very slowly drop-by drop pass 1 ml Elution Buffer through the Lentivirus unit and collect in the tube containing the buffer.

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.



Leave the syringe (with Elution buffer remaining in it), attached to the LentiSelect unit and incubate for 5 - 10 min at room temperature. Pass the remaining Elution buffer through the LentiSelect unit very slowly as before.

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 infectivity.

Transfer 14 ml of the eluate/buffer mixture to each of 2x Vivaspin 20 units. Place the Vivaspin 20's into a centrifuge. 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 xg in a swing-out rotor, or 6,000 xg in a 25° fixed-angle rotor, with cavities accepting 50 ml conical bottom tubes.

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

Caution: Do not reduce the volumes to less than 0.5 ml each in order to avoid aggregation and loss of infectivity.

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.

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 2-6 x 10⁸ viral particles in 3 ml eluate. You can achieve higher titres by concentration in the supplied Vivaspins.

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