LV Mini Purification Kit

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Introduction

Traditionally the recombinant lentivirus is purified by ultracentrifugation to separate the virus particles from cellular proteins and media components. The ultracentrifugation procedure is time consuming and limited to the amount of cell lysate to be processed, in addition, the ultracentrifugation also concentrates cellular debris, membrane fragments, and unwanted proteins from culture medium.

The ViraTrap™ lentivirus mini purification kit is designed for fast and efficient purification of recombinant lentiviruses from lentiviral-transfected cell culture supernatant. Up to $2x10^7$ viral particles can be purified from cell culture media of 1 to 2 T75 flasks. The viruses are first pelleted from viral supernatant and then further purified and concentrated through a purification column and a desalting/concentration unit.

Each column can be regenerated for purifying the same lentivirus. For optimized viral binding and recovery, each column can be regenerated only once.

Before Starting

Familiar with each step by reading this menu and prepare all materials for the procedure.

Kit Components

Catalog#	V1170-00	V1170-01	V1170-02
Preps	1	10	20
LV Columns	1	5	10
Press-On Cap	1	10	20
*Centrifugal filter	1	5	10
15 mL Conical Tube	2	10	20
Buffer P	10 mL	50 mL	100 mL
Buffer LS	5 mL	25 mL	50 mL
Buffer MS	5 mL	25 mL	50 mL
Regeneration Buffer	5	30 mL	50 mL

*Centrifugal device (CF01) can be purchased from Biomiga separately.

Stability

The Guaranteed shelf life is 12 months from the date of purchase. Store LV column at 4°C and all other components at room temperature.

Safety considerations

The lentiviral infected cell culture and the purified virus can be potential bio-hazardous material and can be infectious to human and animals. All protocols MUST be performed under at least Bio-Safety level 2 working condition.

Materials required but not supplied

- Standard TC centrifuge
- Swing bucket rotor
- 0.45 μm filter unit
- Rack holder for column
- PBS

Harvest Lentivirs-Infected Culture

1. Centrifuge the lentivirus-infected culture media at 3,000 rpm for 10 minutes at 4°C. Filter the supernatant through a 0.45**μM** filter. Supernatant from 1 - 2 T75, up to 30 mL of supernatant, can be processed per prep.

   Note: The supernatant can also be stored at -80°C for future purification.

Concentration of lentivirus

2. Add 1 volume of Buffer P to 3 volume of virus supernatant (For example, add 5 mL of Buffer P to 15 mL of virus supernatant). Mix well and incubate at 4°C overnight. The virus is stable in Buffer P.

3. Centrifuge the sample at 6,000 rpm for 30 minutes at 4°C (Proceed to step 4 during centrifugation). Carefully aspirate the supernatant. Spin briefly and remove the residual supernatant. The virus containing pellet should be visible. The pellet may appear hazy. Keep the virus on ice and proceed to step “5”.

Purification column preparation

4. Inverting the LV column to resuspend the resin. Put the column into a 15 mL conical tube and centrifuge at 600xg for 2-5 min. Tear off the breakoff tip on the bottom of the column and place the column into the 15 mL tube. Loosen the cap to allow buffer drain out from the column by gravity. Once the liquid stops dripping, add 5 mL of Buffer LS evenly to the column and let it drain out by gravity without drying the column out.
   Note: A press on cap for the bottom tip of the column is provided for stopping the gravity flow at any time.

5. Resuspend the pellet from step “3” with 300 µL of Buffer LS. Dissolve the pellet by pipetting and transfer the sample to a 1.5 mL eppendorf tube. Spin the sample at 3000 rpm at 4°C for 2 min, transfer the clear supernatant to a clean tube. Keep the virus on ice.

   Note: The virus is ready for infecting cell lines and other *in vitro* applications. For in vivo study and other downstream applications that require higher purities, proceed to step 6.

Load the sample to the purification column

6. Slowly apply the sample from step 5 evenly, dropwise, to the LV column from step 4. Once the entire sample gets into the matrix, proceed to next step. Do not let the column dry out.

Elute Lentivirus from the purification column

7. Add 3 mL of Buffer MS evenly to the column and collect 3 mL of the flow-through. The virus is in the flow through liquid.

Concentration

8. Apply the entire sample collected from step 7 to the reservoir of a centrifugal filter and centrifuge at 3,000 rpm for 15 -20 min at 4°C till approximately 500-1000 µL remains in the reservoir. Pipet the solution up and down several times in reservoir and transfer the virus containing solution a clean vial. The purified virus is ready for downstream applications.

   Note: A swing bucket rotor is preferred. Fixed angle rotor requires higher speed of 7000 rpm for 15-20 minutes.

   Note: Time for centrifugation may vary for different type rotors. Always centrifuge less time and check the liquid level, repeat centrifuge to get to the expected volume. Don’t let the sample volume go below 500 µL.

9. Aliquot and store the purified virus at -80°C. Before infect target cells, we recommend adding the needed amount of purified virus to 5-10 mL culture medium of your target cells and filter through a 0.2 µm sterile filter before infection.

- Typical concentration volume Vs. spin time (Swing bucket rotor, 3,000 rpm at RT, 4 mL starting volume) for 100K centrifugal filter device

  Spin time-15 min: concentrate volume 176 µL
  Spin time-20 min: concentrate volume 76 µL
  Spin time-25 min: concentrate volume 58 µL

- Typical concentration volume Vs. spin time (35º Fixed angle rotor , 7000 rpm RT, 4 mL starting volume) for 100K centrifugal filter device

  Spin time-10 min: concentrate volume 97 µL
  Spin time-15 min: concentrate volume 54 µL
  Spin time-20 min: concentrate volume 35 µL

Regeneration of the column

Upon completion of the purification, add 5 mL of Regeneration Buffer to the column and let the buffer passes through the column by gravity flow. Wash the column by 2x5 mL of PBS, let the PBS pass through the column by gravity flow. Once the liquid stops dripping, fill the column with 4 ml of PBS. Press on the cap to the bottom. Screw on the cap and wrap the column with parafilm in a zip block bag and store at 4℃.

Problems	Solutions
Slow flow rate caused by air bubbles in the resin bed	• Cap the bottom of the column with the press on cap and spin the column at 300 x g for 5 minutes.
Slow flow rate caused by invisible bubbles	• With the bottom cap on, add degassed water to the resin with a height of 1-2 cm of the solution. • Place the entire bottom-capped column in a 15 mL conical tube and centrifuge at 10 minutes at 600 x g.
Supernatant very viscous	• Forgot to filter the supernatant through a 0.45 µM filter unit.
Column clogged after loading sample	• Resuspend and dissolve the virus pellet completely with Buffer S. Spin down briefly to remove any insoluble debris.

Limited Use and Warranty

This product is intended for in vitro research use only. Not for use in human.

This product is warranted to perform as described in its labeling and in Biomiga's literature when used in accordance with instructions. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Biomiga. Biomiga's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Biomiga, to replace the products, Biomiga shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

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