Table of Contents

V281001

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 purification maxi kit is designed for fast and efficient purification of recombinant lentiviruses from lentiviral-transfected cell culture supernatant. Viral particles can be purified from cell culture of 4 to 5 T75 flasks per column. The viruses are first applied to a purification column and then further purified and concentrated through a 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#	V1270-00	V1270-01	V1270-02
Preps	1	4	10
LV Columns	1	2	5
Press-On Cap	1	4	10
Centrifugal Device	1	4	10
50 mL Conical Tube	2	4	10
Buffer VP	10 mL	80 mL	180 mL
Buffer VS	5 mL	40 mL	90 mL
Buffer MS	5 mL	40 mL	90 mL
Regeneration buffer	10 mL	20 mL	45 mL

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

Harvest LV infected cell culture (for 4-5 T75 flasks)

1. Centrifuge the LV culture media at 3,000 rpm for 10 min at 4°C and filter through a 0.45 µM filter unit. Transfer the supernatant into a clean tube and add 1 volume of Buffer VP to 3 volume of supernatant (for example, add 10 mL of Buffer VP to 30 mL of supernatant). Mix well and incubate at 4°C for at least 3 hours to overnight. The virus is stable in Buffer P for up to 1 week.

2. Centrifuge the samples at 6,000 rpm for 30 minutes at 4°C (Proceed to step 3 during centrifugation). Carefully aspirate the supernatant. Spin briefly and remove the residual supernatant. Take great care not to disturb the pellet. The virus containing pellet should be visible and may appear hazy. Keep the virus on ice and proceed to step “4”.

Column preparation

3. Inverting and shaking the LV column to resuspend the resin inside the column. Put the column into a 50 mL conical tube and centrifuge at 600xg for 2 min. Tear off the breakoff tip on the bottom of the column and place the column into the 50 mL tube. Loosen the cap to allow buffer drain out from the column by gravity. Once the liquid stops dripping, add 6 mL of Buffer VS 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.

4. Resuspend pellet from step “2” completely with 4 mL Buffer VS. Spin the sample at 3,000 rpm at 4°Cfor 5 min and transfer the supernatant to a clean vial. Keep the virus on ice.

5. Apply the sample to a centrifugal filter and spin at 3,000 rpm for 15-20 min at 4°C till approximately 500 µL of sample remains in the reservoir.

Load the sample to the purification column

6. Transfer the sample from step “5” evenly to the LV column and let it flow into resin by gravity. Once the entire sample gets into the resin, proceed to the next step.

   Note: Slowly add the sample dropwise to the resin. Once the entire sample gets into the matrix, proceed to next step. Do not let the column dry out.

Elute LV from the purification column

7. Add 6 mL of Buffer MS evenly to the LV column and collect 6 mL of the flow-through. The virus is in the flow through liquid. Keep the virus on ice.

Concentration

8. Apply 4 mL sample collected from step 7 to the reservoir of a centrifugal filter and centrifuge at 3,000 rpm for 10 min at 4°C, process the remaining sample as described. Continue to spin the sample at 3,000 rpm for 10-15 minutes at 4°C till approximately 1000 µL remains in the reservoir. Pipet the solution up and down several times in reservoir and transfer the virus containing solution to a clean vial. The purified virus is ready for downstream applications.

   Note: Always centrifuge less time and check the liquid level, do not let the solution dry out. Continue to centrifuge till the desired volume is achieved.

   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 of rotors.

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.

Regeneration of the column

10. Upon completion of the purification procedure, add 5 *mL* of **Regeneration Buffer** to the column and let the buffer passes through the column by gravity flow. Wash the column by 10 *mL* of **PBS,** let the PBS pass through the column by gravity flow. Once the liquid stops dripping, fill the column with 3-5 ml of PBS. Press the press-on cap to the bottom. Screw the cap and wrap the column with parafilm in a zip block bag and store at 4°C.

- 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

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.
Cell pellet very viscous	• Filter the supernatant through a 0.45 µM filter unit.
Column clogged after loading sample	• Spin down briefly to remove any insoluble debris before loading to column. Resuspend and dissolve the virus pellet completely with Buffer S before loading to column.

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.

For technical support or learn more product information, please contact us at (858) 597-0602 or visit our website at www.biomiga.com