EasyPrep™ Lentivirus Purification Maxi Kit Manual

Catalog# LV02-00, LV02-01, LV02-02

Bioland

For research use only

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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 EasyPrep™ lentivirus maxi purification 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#	LV02-00	LV02-01	LV02-02	Storage
Preps	2	4	10	4°C
LV Columns	1	2	5	RT
Press-On Cap	2	4	10	RT
Centrifugal Filters	2	4	10	4°C
50mL Conical Tube	2	4	10	RT
Buffer P	10 mL	80 mL	180 mL	RT
Buffer S	5 mL	40 mL	90 mL	RT
Buffer MS	5 mL	40 mL	90 mL	RT
Regeneration Buffer	10 mL	20 mL	45 mL	RT

Stability

The Guaranteed shelf life is 12 months from the date of purchase. Store LV column at 4°C

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

Lentivirus Purification Maxi Kit

1. Harvest Lentivirus production culture medium. Centrifuge the medium at 3,000 rpm for 10 minutes. Filter the supernatant through a 0.45 micron filter. *Supernatant from 4 - 5 T_{75} flasks can be processed per preparation.*

2. Add 1 volume Buffer P to 3 volume of virus supernatant (For example, add 10 mL Buffer P to 30 mL of virus 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.

3. Centrifuge the sample at 6,000rpm 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 and may appear hazy. Keep the virus on ice and proceed to Step 5.

4. **Column Preparation:** Inverting and shaking the LV column to resuspend the resin inside the column. Put the column into a 50mL conical tube and centrifuge at 600xg for 2 minutes. 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 Buffer S evenly to the column and let it drain out by gravity without drying the column.

   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 4 mL Buffer S. Dissolve the sample completely by pipetting. Spin the sample at 3,000 rpm at 4°C for 5 min.

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

7. Apply the sample evenly to the LV column and let it flow into resin by gravity. Once the entire sample gets into the resin, proceed to 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.

8. Add 6 mL 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

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

    - *A swing bucket rotor is preferred. Fixed angle rotor requires higher speed of 7000 rpm for 15-20 minutes. Time for centrifugation may vary for different type of rotors.*
    - *Always centrifuge less time and check the liquid level, repeat centrifuge to get to the expected volume.*

Swing Bucket Rotor (3,000 rpm, 4 mL starting volume)		**35° Fixed Angle Rotor** (7000 rpm, 4 mL starting volume)	
Spin time	Volume left	Spin time	Volume left
15 min	**176 µL**	10 min	**97 µL**
20 min	**76 µL**	15 min	**54 µL**
25 min	**58 µL**	20 min	**35 µL**

10. (Optional) If a special buffer is needed for downstream applications, the buffer can be exchanged. Add PBS or other desired buffer up to the 4 mL mark on the column and spin at 4000 × g for desired time (see above). Transfer the virus containing solution to a clean vial. The purified virus is ready for downstream applications.

11. In 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

12. Upon completion of the purification, add 5 mL Regeneration Buffer to the column and let the buffer passes through the column by gravity flow. Wash the column by 10 mL PBS, let the PBS pass through the column by gravity flow. Once the liquid stops dripping, fill the column with 3-5 ml 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°C.

Trouble Shooting

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 1,000 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.

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