EasyPrep™ Lentivirus Purification Mini Kit Manual

Catalog# LV01-00, LV01-01, LV01-02

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 mini purification kit is designed for fast and efficient purification of recombinant lentiviruses from Lentiviral transfected cell culture supernatant. Up to $3x10^{10}$ viral particles can be purified from cell culture media of 1 to 2 T_{75} 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#	LV01-00	LV01-01	LV01-02
Preps	2	10	20
LV Columns	1	5	10
Press-On Cap	1	5	10
Desalting Filter	2	5	10
15 mL Conical Tube	2	5	10
Buffer P	10 mL	50 mL	100 mL
Buffer S	5 mL	25 mL	50 mL
Buffer MS	5 mL	25 mL	50 mL
Regeneration Buffer	5 mL	30 mL	50 mL

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
- PBS

Lentivirus Purification Mini 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 1 - 2 T_{75} flasks (up to 30 mL) can be processed per preparation.*
   - *The supernatant can also be stored at -80°C for future purification.*
2. Add 1 volume Buffer P to 4 volume of virus supernatant (For example, add 5 mL Buffer P to 20 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.
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.
4. Inverting and shaking the LV column to resuspend the resin inside the column. Put the column into a 15 mL conical tube and centrifuge at 800 rpm for 2 minutes. 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 Buffer S 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 Buffer S. Dissolve the sample completely by pipetting. Keep the virus on ice.

6. Apply the sample evenly to the column and let it pass through the column by gravity. Discard the flowthrough liquid in the collection tube.

   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.

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

Concentrating and Buffer exchanging

Place the desalting column into a 15 ml conical tube. Add up to 4 mL of sample (up to 3.5 mL if using a 23 degree fixed angle rotor) onto the filter column. Cap the conical tube.

8. Place the conical tube into the centrifuge rotor (swinging bucket preferred), counterbalance with a same weight tube.

9. Spin at 3,000 rpm for 15 -20 minutes till 500 –1000 **µ**L 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.

   - *If use fixed angle rotor, spin at 7000 rpm for 15-20 minutes.*
   - *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 500ul.*

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. If a special buffer is needed for downstream applications, the buffer can be exchanged. Add 1xPBS or other desired buffer up to the 4 mL mark on the column and spin at 4000 × g for desired time (see table above). The viruses are now in the residual buffer at the bottom of the column.

11. Aliquot and store the purified virus at -80°C. Before infect the target cells, it's recommended to add the needed amount of the purified virus to 5-10 ml culture medium and filter through a 0.2µm sterile filter.

12. Store the desalting filter column in a plastic bag at 4°C for use of the same virus.

Regeneration of the column

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 5mL 1xPBS two times, let the PBS pass through the column by gravity flow. Once the liquid stops dripping, fill the column with 4ml 1xPBS. 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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Bioland Scientific LLC

14925 Paramount Blvd., Suite C

Paramount, CA 90723

USA

Tel: (877) 603-8882

Fax: (562) 733-6008

Email: service@bioland-sci.com

order@bioland-sci.com

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