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Lentivirus Mini Purification Kit

(Cat# K1305-10, -20; Store at 4 °C)

I. 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.

BioVision's Lentivirus (LV) 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 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.

II. Sample Types:

For fast and efficient purification of recombinant Lentiviruses from Lentiviral transfected cell culture supernatant

III. Kit Contents:

Components	K1305-10 10 preparations	K1305-20 20 preparations	Part Number
LV Mini Columns	5	10	K1305-XX-1
Press-On Caps	10	20	K1305-XX-2
Centrifugal Filters	5	10	K1305-XX-3
15 mL Conical Tube	5	10	K1305-XX-4
Buffer P	50 mL	100 mL	K1305-XX-5
Buffer S	25 mL	50 mL	K1305-XX-6
Buffer MS	25 mL	50 mL	K1305-XX-7
Regeneration Buffer	30 mL	50 mL	K1305-XX-8

IV. User Supplied Reagents and Equipment:

- ddH_2O
- Standard TC centrifuge
- Swing bucket rotor
- 0.45 µm filter unit
- Rack holder for column
- PBS

V. Shipment and Storage:

All the reagents are shipped at 4°C. Except the LV mini columns, which is stored at 4°C, all other components are stored at room temperature. The guaranteed shelf life is 12 months from the date of purchase. DO NOT FREEZE!

VI. Lentivirus Purification Protocol:

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

1. Harvest Lentivirus infected culture:

a. Centrifuge the Lentivirus-infected culture media at 3,000 rpm for 10 min 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.*

2. Concentration of Lentivirus:

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

b. Centrifuge the sample at 6,000 rpm for 30 min at 4 °C (Proceed to step 3 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 next step.

3. Purification column preparation:

a. Invert and shake the LV column to resuspend the resin inside the column. Put the column into a 15 mL conical tube and centrifuge at 600 g 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 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.*

b. Resuspend the pellet with **300 µL of Buffer S**. Dissolve the pellet by pipetting and transfer the sample to a 1.5 ml eppendorf tube. Spin the sample at 3,000 rpm at 4 °C for 2 min and transfer the clear lysate 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 next step

4. Load the sample to the purification column: a. Slowly apply the sample evenly, dropwise to the column (from 3b) and let it pass through the column by gravity. Discard the flow through 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.

5. Elute Lentivirus from the purification column:

a. 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.

6. Concentration:

a. Apply the entire sample collected to the reservoir of a centrifugal filter and centrifuge at 3,000 rpm for 15-20 min till **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 min. 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**.

b. Aliquot and store the purified virus at -80 °C

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

I. Spin time-15 min: concentrate volume 176 µL
II. Spin time-20 min: concentrate volume 76 µL
III. Spin time-25 min: concentrate volume 58 µL

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

I. Spin time-10 min: concentrate volume 97 µL
II. Spin time-15 min: concentrate volume 54 µL
III. Spin time-20 min: concentrate volume 35 µL

7. 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 **2 X 5 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 °C.

VII. Related Products:

Product Name/Catalog Number
Adenovirus Mini Purification Kit # K1300-10, -20
Adenovirus Maxi Purification Kit # K1301-2, -4, -10
Adeno-associated Virus Mini Purification Kit # K1302-10, -20
Adeno-associated Virus Maxi Purification Kit # K1303-2, -4, -10
Adeno-associated Virus Mini Purification Kit, all serotypes # K1304-10, -20
Adeno-associated Virus Maxi Purification Kit, all serotypes # K1311-2, -4, -10
Lentivirus Mini Purification Kit # K1305-10, -50
Lentivirus Maxi Purification Kit # K1306-2, -4, -10
Retrovirus Mini Purification Kit # K1307-10, -20
Retrovirus Maxi Purification Kit # K1308-2, -4, -10
HCV Mini Purification Kit # K1309-10, -20
HCV Maxi Purification Kit # K1310-2, -4, -10

VIII. General Troubleshooting Guide:

Problems	Solution
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 300g for 5 min
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 min at 1,000g
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

FOR RESEARCH USE ONLY! Not to be used on humans.