Lentivirus Maxi Purification Kit

(Cat# K1306-2, -4, -10, Shipped 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) 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.

II. Sample Type:

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

III. Kit Contents:

	K1306-2	K1306-4	K1306-10		
	2 Preparations	**4 Preparations**	**10 Preparations**	**Part Number**	**Storage Temp**
LV Maxi Columns	1	2	5	K1306-XX-1	4°C
Press-On Cap	2	4	10	K1306-XX-2	RT
Centrifugal Filters	2	4	10	K1306-XX-3	RT
50 mL Conical Tube	1	2	5	K1306-XX-4	RT
Buffer P	20 mL	80 mL	180 mL	K1306-XX-5	RT
Buffer S	20 mL	40 mL	90 mL	K1306-XX-6	RT
Buffer MS	15 mL	40 mL	90 mL	K1306-XX-7	RT
Regeneration Buffer	15 mL	20 mL	40 mL	K1306-XX-8	RT

IV. User Supplied Reagents and Equipment:

- ddH_2O
- Standard TC centrifuge
- Swinging bucket rotor
- 0.45 µm filter unit
- Rack holder for column
- PBS
- 50 mL Conical Tubes

V. Shipment and Storage:

All the reagents are shipped at room temperature (RT). Except the LV columns, which are stored at 4°C, all other components are stored at RT. 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 LV culture media at 3,000 rpm for 10 min and filter through a 0.45 µM filter unit. Transfer the supernatant into a clean tube and add 1 volume of **Buffer P** to 4 volumes of supernatant (for example, add 10 mL of **Buffer P** to 40 mL of supernatant). Mix well and incubate at 4°C for at least 3 hr to overnight. The virus is stable in **Buffer P** for up to 1 week.

2. Centrifuge the samples at 6,000 rpm for 30 min. 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 next step.

3. Column preparation:

a. Invert and shake the LV column to resuspend the resin inside the column. Put the column into a 50 mL conical tube and centrifuge at 500 rpm 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 **5.5 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. The LV maxi columns can be used twice.*

b. Resuspend the pellet from "step 2" completely with **3.5 mL Buffer S.** Spin the sample at 3,000 rpm at 4°C for 5 min and transfer the supernatant to a clean vial. Keep the virus on ice.

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

4. Load the sample to the purification column:

a. Transfer the sample evenly to the 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 out.*

5. Elute Lentivirus from the purification column:

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

6. Concentration:

a. Apply 4 mL sample collected from step 5a 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 min at 4°C till 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 swinging 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 of rotors.

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

7. Regeneration of the column:

Upon completion of the purification procedure, add **5 mL of Regeneration Buffer** to the column and let the buffer pass through the column by gravity flow. Wash the column with **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 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.

- Typical Concentration Volume vs. Spin Time (Swinging bucket rotor, 3,000g 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° Fixed angle rotor, 7000 rpm at 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

VII. Related Products:

Products/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	• 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 the column.

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