

## ab288111 – Lentivirus Maxi Purification Kit

For the purification of recombinant Lentiviruses from Lentiviral transfected cell culture supernatants

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab288111>

### Storage and Stability

- Excluding the LV columns, which are stored at 4°C, all other components are stored at RT. Do not freeze.
- The shelf life is 12 months from the date of purchase

### Materials Supplied

Item	Quantity			Storage Condition
	2 Preparation kit	4 Preparation Kit	10 Preparation Kit	
LV Maxi Columns	1	2	5	4°C
Press-On Caps	2	4	10	RT
Centrifugal Filters	2	4	10	RT
50 mL Conical Tube	1	2	5	RT
Buffer P	20 mL	80 mL	180 mL	RT
Buffer S	20 mL	40 mL	90 mL	RT
Buffer MS	15 mL	40 mL	90 mL	RT
Regeneration Buffer	15 mL	20 mL	40 mL	RT

### Materials Required, Not Supplied

- ddH<sub>2</sub>O
- Standard TC centrifuge
- 0.45 µm filter unit
- Rack holder for column
- PBS
- 50mL conical tubes

### Reagent Preparation

- Read the entire protocol before performing the experiment.
- 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

### Protocol

1. Harvest Lentivirus infected culture:  
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. Centrifugation  
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 the 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.  
**ΔNote:** 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:  
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.  
**ΔNote:** 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  
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 5 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.  
**ΔNote:** 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.  
**ΔNote:** 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:

- Spin time-15 min: concentrate volume 176  $\mu$ L
- Spin time-20 min: concentrate volume 76  $\mu$ L
- Spin time-25 min: concentrate volume 58  $\mu$ L

Typical Concentration Volume vs. Spin Time (35° Fixed angle rotor, 7000 rpm at RT, 4 mL starting volume) for 100K centrifugal filter device:

- Spin time-10 min: concentrate volume 97  $\mu$ L
- Spin time-15 min: concentrate volume 54  $\mu$ L

### **Technical Support**

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