Retrovirus Maxi Purification Kit

(Cat# K1308-2, -4, -10, Shipping at RT; Storage at Multiple Temperatures)

I. Introduction:

BioVision's Retrovirus Maxi Purification Kit is designed for fast and efficient purification of recombinant Retroviruses from Retrovirus transfected cell culture supernatant. Up to $1x10^7$ viral particles can be purified from cell culture media of 5 to 6 T75 flasks. Traditionally, the recombinant Retrovirus (RV) is purified by ultracentrifugation using CsCl to separate the virus particles from cellular proteins and media components. The CsCl ultracentrifugation procedure is time consuming and limited to the amount of cell lysate to be processed. Each column can be regenerated for purifying the same Retrovirus. For optimized viral binding and recovery, each column can be regenerated only once.

II. Sample Type:

For fast and efficient purification of recombinant Retroviruses from Retrovirus transfected cell supernatants

III. Kit Contents:

	K1308-2	K1308-4	K1308-10		Storage
	2 Preparations	4 Preparations	10 Preparations	Part Number	Temperature
RV Maxi Columns	1	2	5	K1308-XX-1	4°C
Press-On Caps	1	2	10	K1308-XX-2	RT
Centrifugal Filters	2	4	10	K1308-XX-3	RT
50 mL Collection Tube*	2	4	10	K1308-XX-4	RT
10X RV Wash Buffer	20 mL	40 mL	80 mL	K1308-XX-5	RT
2X RV Elution Buffer	20 mL	40 mL	80 mL	K1308-XX-6	RT
Regeneration Buffer	15 mL	30 mL	50 mL	K1308-XX-7	RT

**One of the 50 mL Collection tube(s) is the tube in which the Maxi column(s) is provided. Please use it as mentioned in the protocol.*

IV. User Supplied Reagents and Equipment:

- ddH_2O
- 0.45 µm filter unit
- 0.22 µm syringe filter
- Rack holder for columns
- PBS
- 15 mL & 50 mL Conical Tubes

V. Shipment and Storage:

All the reagents are shipped at room temperature (RT). Except the RV Maxi 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. Retrovirus Purification Protocol:

The Retrovirus infected cell media 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 working condition.

1. Harvesting supernatant from Retrovirus infected cells (5-6 T75 flask or equivalent per column):

a. Centrifuge the Retrovirus infected culture media at 3,000 rpm for 10 min at 4°C. Filter the supernatant through a **0.45 µm filter unit**. *Note: Supernatant from 5-6 T75 flasks can be processed per column. Up to $1x10^7$ virus particles can be purified per column.*

b. The supernatant is ready for purification. *Note: the supernatant can also be stored at -80°C for future purification.*

2. Equilibrate the RV Maxi Column:

a. Dilute the 10X RV Wash Buffer with ddH_2O to 1X Wash Buffer.

b. Dilute the 2X RV Elution Buffer with ddH_2O to 1X Elution Buffer.

c. Set the column in a **50 mL Collection Tube** and spin at 600g for 2 min. Hold the column with a clamp or other holders. Twist off the bottom and let the liquid drop by gravity flow. Equilibrate the column with **4 mL** of ddH_2O and then **10 mL 1X Wash Buffer.**

Notes:

- *Centrifugation can help remove the bubbles created during shipping.*
- *A swing-bucket rotor is preferred for centrifugation.*
- *If the flow-through is too slow, alternative is to set the column in a fresh 50 mL* Conical Tube *and centrifuge at 600g for 1 min.*
- *If the flow-through is too slow, make sure to remove any visible bubbles (see troubleshooting guide).*
- *There is a Press-On Cap supplied in the kit for the bottom of the column to stop the flow.*

3. Load the Retrovirus containing supernatant to the RV Maxi Column:

a. Load 15 mL of the supernatant to the RV Maxi Column and let the supernatant gradually run through the column. Keep loading till all samples pass through the column**. Optional:** Collect the flow through and reload to the same column one more time to ensure maximal viral particle binding.

Note: If the gravity flow through rate gets noticeably slow during loading or reloading of the supernatant, set the Column in a 50 mL Conical Tube and centrifuge at 600g for 2-5 min at 4°C. Note: Load 15 mL of supernatant to column each time.

4. **Wash the column and elute the Retrovirus from the purification column:**
   a. Wash the column with **10 mL Wash Buffer**. Repeat once. This step can be performed either by gravity flow or centrifugation at 600g for 5 min at 4°C.
   b. Elute the virus by applying **4 mL Elution Buffer**. Collect the **4 mL flow through** in a fresh Conical Tube (not provided).
5. **Desalting and Buffer exchange:**
   a. Apply **4 mL** of the sample collected from the step above to the reservoir of a **Centrifugal Filter** and centrifuge at 3,000 rpm for 5-10 min at 4°C till 500 µL remains in the reservoir. Fill the reservoir to 4 mL with 3.5 ml PBS and centrifuge at 3,000 rpm for 10-15 min at 4°C till 500 µL remains in the reservoir. Pipet the solution up and down several times in the reservoir and transfer the virus containing solution to a clean 50 mL Conical Tube (not provided). *Note: A swing bucket rotor is preferred. Fixed angle rotor requires higher speed of 7000 rpm for 20 min. See typical concentration volume Vs. spin time below. Note: If not using the centrifugal device, the virus can also be desalted by dialysis or other desalting columns. Note: Time for centrifugation may vary for different type of rotors. Always centrifuge for lesser time and check the liquid level, repeat centrifugation to get to the expected volume.*
6. 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 0.22 µm sterile filter before infection.
7. **Regeneration of the column:**
   a. Upon completion of the purification, add **8 mL of Regeneration Buffer** to the Maxi Column. Let the buffer run through the column by gravity flow and then add **5 mL** of 1X Wash Buffer. Press on the cap to the bottom. Wrap the column with parafilm in a zip block bag and store at 4°C.

- Typical concentration volume vs. spin time (Swing bucket rotor, 3,000g at RT, 4 mL starting volume) for 100K centrifugal filter device:
  I. Spin time-10 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 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 (Cat. No. K1300-10, -20)
Adenovirus Maxi Purification Kit (Cat. No. K1301-2, -4, -10)
Adeno-associated Virus Mini Purification Kit (Cat. No. K1302-10, -20)
Adeno-associated Virus Maxi Purification Kit (Cat. No. K1303-2, -4, -10)
Adeno-associated Virus Mini Purification Kit, all serotypes (Cat. No. K1304-10, -20)
Adeno-associated Virus Maxi Purification Kit, all serotypes (Cat. No.K1311-2, -4, -10)
Lentivirus Mini Purification Kit (Cat. No. K1305-10, -50)
Lentivirus Maxi Purification Kit (Cat. No. K1306-2, -4, -10)
Retrovirus Mini Purification Kit (Cat. No. K1307-10, -20)
Retrovirus Maxi Purification Kit (Cat. No. K1308-2, -4, -10)
HCV Mini Purification Kit (Cat. No. K1309-10, -20)
HCV Maxi Purification Kit (Cat. No. K1310-2, -4, -10)

VIII. General Troubleshooting Guide:

Problems	Solution
Slow flow rate caused by air bubbles in the resin bed	• Cap the column bottom and add water so that the resin is covered by a height of 1-2 cm of solution • Stir the resin with a clean spatula or Pasteur pipette, until all portions of the resin are loosely suspended in the solution. • With the bottom cap on, let the column stand for 5 min until the resin settles.
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.
Cell line didn't survive after infection of the purified virus	• Dialyze the purified virus to PBS or desired buffer before infecting cell lines. • Use desalting column and perform buffer exchange.

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