ab288091 – Retrovirus Maxi Purification Kit

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

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab288091

Storage and Stability

- All the reagents are shipped at room temperature. Except the RV maxi columns, which are 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!

Materials Supplied

Item	Quantity			Storage Condition
	2 Preparation kit	4 Preparation Kit	10 Preparation Kit	
RV Maxi Columns	1	2	5	4°C
Press-On Caps	1	2	10	RT
Centrifugal Filters	2	4	10	RT
10X RV Wash Buffer	20 mL	40 mL	80 mL	RT
2X RV Elution Buffer	20 mL	40 mL	80 mL	RT
Regeneration Buffer	15 mL	30 mL	50 mL	RT
50 mL Collection tubes	2	4	10	RT

∆**Note:** *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.*

Materials Required, Not Supplied

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

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 (BSL2) working condition.

1. Harvest supernatant from Retrovirus infected cells (For five to six 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 five to six T75 flasks can be processed 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 Columns:
   a. Dilute the 10X Wash Buffer with ddH_2O to 1X Wash Buffer.
   b. Dilute the 2X Elution Buffer with ddH_2O to 1X Elution Buffer.
   c. Set the Maxi 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 ddH2O 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, the other alternative is to set the column in a 50 mL conical tube and centrifuge at 600g for 1 min.*
   - *There's a press-on cap supplied in the kit for the column tip to stop the flow.*
   - *If the flow-through is too slow, make sure to remove any visible bubbles.*

3. Load the RV -containing supernatant to the Maxi Column
   a. Load the supernatant to the Maxi Column and let the supernatant gradually run through the column. Collect the flow through and reload to the same column one more time to ensure maximal 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 RV Wash Buffer. Repeat once. This step can be performed either by gravity flow or centrifugation at 600g for 5 min.
   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 less time and check the liquid level, repeat centrifuge to get to the expected volume.*

6. Aliquot and store the purified virus at -80°C. Before infecting target cells, it is recommended to add 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

Technical Support

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