AV Maxi Purification Kit

Introduction

The ViraTrap™ adenovirus purification maxi kit is designed for fast and efficient purification of recombinant adenoviruses from retroviral-transfected cell culture supernatant. Up to $1x10^{11}$ viral particles can be purified from cell culture media of five to six T75 flasks.

Traditionally the recombinant adenovirus is purified by ultra centrifugation 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 adenovirus. For optimized viral binding and recovery, each column can be regenerated only once.

Before Starting

Familiar with each step by reading this menu and prepare all materials for the procedure.

Kit Components

Catalog#	V1260-00	V1260-01	V1260-02	Notes
Preps	1	4	10	
AV Maxi Columns	1	2	5	Store @ 4°C
Centrifugal Filter*	1	2	5	Store at RT
Press-On Cap	1	4	10	Store at RT
50 mL Collection Tube	1	4	10	Store at RT
10x AV Wash Buffer	10 mL	40 mL	80 mL	Store at RT
2x AV Elution Buffer	10 mL	40 mL	80 mL	Store at RT
Regeneration Buffer	10 mL	30 mL	50 mL	Store at RT

*Centrifugal filter can be purchased from Biomiga separately.

Stability

The guaranteed shelf life is 12 months from the date of purchase.

Safety considerations

The adenovirus infected cell media and the purified virus can be potential bio-hazardous material and can be infectious to human and animals. All protocols MUST be performed under at least Bio-Safety level 2 working condition.

Materials required but not supplied

- ddH_2O
- PBS
- 0.45 µm filter unit and 0.22 µm syringe filter
- Rack holder for column

Harvesting supernatant from adenovirus-infected cells (For five to six T75 flask or equivalent per column)

1. For a T75 flask, transfer 8 mL of supernatant to a clean 50 mL tube, leave around 3 mL of supernatant. Collect the cells by a scraper and transfer the lysate to another 50 mL tube.
   Combine all supernatant to the 50 mL tube and proceed to Step 3. Combine all cell lysate to the 50 mL tube and proceed to Step 2.

2. Freeze and thaw the cell lysate between 37°C and dry ice/ethanol three times and proceed to Step 3.

3. Centrifuge the sample at 4°C, 3,000 rpm for 10 minutes. Filter the supernatant through a 0.45 µM filter unit. The supernatant is ready for purification.

NOTE: The supernatant can also be stored at -80°C for future purification.

Equilibrate the column

Dilute the 10 x Wash Buffer with dd H_2O to 1 x Wash Buffer.
Dilute the 2 x Elution Buffer with dd H_2O to 1 x Elution Buffer.

4. Set the column in a 50 mL centrifuge tube and spin at 400 x g for 2 minutes. Hold the column with a clamp or other holders. Twist off the bottom, loosen the cap, and let the liquid drop by gravity flow. Equilibrate the column with 4 mL of dd H_2O and then 10 mL 1 x Wash Buffer.

NOTE:

- The centrifugation can help remove the bubbles created during shipping.
- A swing-bucket rotor is preferred for centrifugation.
- If the flow-through is noticeably slow, set the column in a 50 mL conical tube and centrifuge at 400 x g for 2 minutes.
- There's a press-on cap supplied in the kit for the bottom of the column to stop the flow.
- If the flow-through is too slow, make sure to remove any visible bubbles (See trouble shooting on page 7).

Load the adenovirus-containing supernatant to the column

5. Load 15 mL of the supernatant to the 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 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 tube and centrifuge at 400 x g for 2-5 min.

   NOTE: Load 15 mL of supernatant to column each time.

Wash the column and elute the adenovirus

6. Wash the column with 10 mL Wash Buffer. Repeat once. This step can be performed either by gravity flow or centrifugation at 400 x g for 5 min.

7. Elute the virus by applying 4 mL Elution Buffer. Collect 4 mL flow through.

Desalting and buffer exchange

8. Apply up to 4 mL of the sample collected from step 6 to the reservoir of a centrifugal filter and centrifuge at 3,000 rpm for 5 -10 minutes at 4°C. Discard the flow through. Process the remaining sample if any and centrifuge till approximately 500 **µ**L remains in the reservoir. Discard the flow through and add 3.5 mL of PBS to the reservoir and centrifuge at 3,000 rpm for 10-15 minutes at 4°C till approximately 500 **µ**L remains in the reservoir. Pipet the solution up and down several times in reservoir and transfer the virus containing solution to a clean vial.

   Note: Alternatively, the supernatant can be dialyzed overnight.
   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.

9. Aliquot and store the purified virus at -80°C. Before infect 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.

Regeneration of the column

10. Upon completion of the purification, add 8 mL of Regeneration Buffer to the column by gravity flow and then add 5 mL of Binding Buffer**.** Press on the cap to the bottom. Wrap the column with parafilm in a zip block bag and store at 4℃.

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

  Spin time-15 min: concentrate volume 176 µL
  Spin time-20 min: concentrate volume 76 µL
  Spin time-25 min: concentrate volume 58 µL

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

  Spin time-10 min: concentrate volume 97 µL
  Spin time-15 min: concentrate volume 54 µL
  Spin time-20 min: concentrate volume 35 µL

Problems	Solutions
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 minutes 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 50 mL conical tube and centrifuge at 10 minutes at 400 x g.
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.

Limited use and warranty

This product is intended for *in vitro* research use only. Not for use in human.

This product is warranted to perform as described in its labeling and in Biomiga's literature when used in accordance with instructions. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Biomiga. Biomiga's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Biomiga, to replace the products, Biomiga shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or learn more product information, please contact us at (858) 597-0602 or visit our website at www.biomiga.com