AV Mini Purification Kit

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Introduction

The ViraTrap^{TM} adenovirus purification mini kit is designed for fast and efficient purification of recombinant Adenovirus from adenovirus transfected cell culture supernatant. Up to 3 x10^{10} viral particles can be purified from cell culture media of 1 to 2 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

Familiarize yourself with each step by reading this menu and preparing all of the materials for the procedure.

Kit Components

Catalog#	V1160-00	V1160-01	V1160-02	Notes
Preps	2	10	20	
AV Mini Column	1	5	10	Store at 4°C
Press-On cap	2	5	10	Store at RT
Centrifugal filter	2	10	20	Store at RT
15 mL Collection Tube	1	5	10	Store at RT
10x AV Wash Buffer	5 mL	25 mL	50 mL	Store at RT
2x AV Elution Buffer	5 mL	25 mL	50 mL	Store at RT
Regeneration Buffer	15 mL	75 mL	150 mL	Store at RT

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 and 0.22 μM filters
- Rack holder for column

Harvest supernatant from adenovirus-infected cells (For 1-2 T75 flask or equivalent per column)

1. For a T75 flask, transfer 8 mL of supernatant to a clean 15 mL tube. Leave around 3 ml of supernatant. Collect the cells by a scraper and transfer the cells and the supernatant to a new 15 mL tube. Freeze and thaw the cell lysate between 37°C and dry ice plus ethanol for three times. Combine the cell lysate with the 8 mL supernatant.

2. Centrifuge the sample at 3,000 rpm, 4°C, for 10 minutes. Transfer and filter the supernatant through a 0.45 μM filter unit. The filtered supernatant is ready for purification. It can also be stored at -80°C.

Equilibrate the column

Dilute the 10 x AV Wash Buffer with ddH_2O to 1 x AV Wash Buffer.

Dilute the 2 x AV Elution Buffer with ddH_2O to 1 x AV Elution Buffer.

3. Spin the column with the 15 mL collection tube in a swing bucket rotor at 400 x g for 2 minutes. Hold the column with a clamp or other holders. Twist off the bottom, loose the cap, and let the liquid drop by gravity flow. Equilibrate the column with 2 mL of ddH_2O and then 5 mL 1x AV Wash Buffer.

NOTE:

- 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 15 mL conical tube and centrifuge at 400 x g for 5 minutes.
- 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 (See trouble shooting on page 6).

Load the AV-containing supernatant to the columns

4. Load 5 mL of supernatant to the column and let the supernatant gradually run through the column. Keep loading until all samples pass through the column. Optional: Reload the flow through to the column once for maximal viral particle binding.

   NOTE: If the flow rate gets noticeably slower, cap (the press-on cap to the bottom and the screw cap to the top) and invert the column to mix the supernatant and resin well. Rock the sample for 5 minutes in a shaker platform. Take off the press-on cap and put the column into 15 mL tube. Centrifuge at 400 x g for 2 minutes. Transfer the flow through to another clean tube if reloading is needed. Keep loading the supernatant until all samples pass through the column.

Wash the column and elute the adenovirus

5. Wash the column with 5 mL 1 x AV Wash Buffer. Repeat once. This step can be performed either by gravity flow or centrifugation at 400 x g for 5 minutes.

6. Elute the virus by applying 4 mL 1 x AV Elution Buffer. Collect 4 mL flow through.

Desalting and Buffer exchange

7. 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 (4°C) for 10-15 minutes until approximately 500 µL remains in the reservoir. Discard the flow through and add 3.5 mL PBS to the reservoir and centrifuge at 3,000 rpm for 10-15 minutes until 400-500 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.
   Note: A swing bucket rotor is preferred

8. 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 0.22 µM sterile filter before infection.

Regeneration of the Column

9. Upon completion of the purification, add 5 mL of Regeneration Buffer to the column by gravity flow and then add 5 mL of 1x AV 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, 3,000 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 (35° Fixed angle rotor, 7,000 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

Trouble Shooting Guide

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 15 mL conical tube and centrifuge at 10 minutes at 400 x g.
Supernatant very viscous	• Filter the supernatant through a 0.45 µM filter unit as mentioned in protocol.
Cell line did not survive after infection of the purified virus	• Dialyze the purified virus to PBS or desired buffer before infecting cell lines.

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, expressed 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.

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