Ver:1904

ViraTrap™ Adenovirus Purification Maxiprep Kit

（BW-V1260）

Contents

Kit Contents

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AV Maxi Columns	1	2	5
Press-On Cap	1	2	5
Centrifugal Filters*	2	4	10
50 mL Centrifugal Tubes	1	2	5
10 x AV Wash Buffer	10 mL	20 mL	50 mL
2 x AV Elution Buffer	10 mL	20 mL	50 mL
Regeneration Buffer	15 mL	30 mL	75 mL
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**: Centrifugal Filters (Cat# BW-CF01) can purchased from BEIWO separately.*

Introduction

The ViraTrap™ Adenovirus Purification Maxiprep Kit is designed for fast and efficient purification of recombinant adenovirus from adenovirus transfected cell culture supernatant. Up to 90% viral particles can be purified from cell culture media of 5 to 6 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.

Storage and Stability

The guaranteed shelf life is 12 months from the date of production. AV Maxi Columns should be stored at 4℃, store all other contents at room temperature (15-25℃).

Before Starting

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

Safety Information

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 not Supplied

1. ddH_2O.
2. PBS.
3. 0.45 μm and 0.22 μm filters.
4. Rack holder for columns.

Protocol

I. Harvest supernatant from adenovirus-infected cells (For 5 to 6 T75 flask or equivalent per column)

1. For a T75 flask, transfer 8 mL of supernatant to a clean **50 mL Centrifugal Tube**, leave around 3 mL of supernatant. Collect the cells by a scraper and transfer the supernatant to a new **50 mL Centrifugal Tube**.

   Note: Combine all supernatant together, combine all cell lysate together. Keep sample on ice.

2. Freeze and thaw the cell lysate between the 37°C and dry ice/ethanol for three times.

3. Centrifuge the supernatant and cell lysate at 4°C, 3,000 rpm for 10 min. Transfer and 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.

II. 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**.

4. Spin an **AV Maxi Column** with the **50 mL Centrifugal Tube** in a swing bucket rotor at 4°C, 500 x g for 2 min. Hold the **AV Maxi Column** with a clamp or other holders. Twist off the **AV Maxi Column**'s bottom closure and loosen the cap, let the liquid drop by gravity flow. Equilibrate the **AV Maxi Column** with 4 mL of ddH_2O and then **10 mL 1 x AV Wash Buffer**.

   Note: Centrifugation removes the bubbles created during shipping.

   Note: A swing-bucket rotor is preferred for centrifugation.

   Note: If the flow-through is too slow, the other alternative is to set the column in a 50 mL Centrifugal Tube and centrifuge at 4°C, 500 x g for 2-5 min.

   Note: There is a **Press-On Cap** supplied in the kit for the column tip to stop the flow.

   Note: If the flow-through is too slow, make sure to remove any visible bubbles (See Trouble Shooting Guide on page 7).

III. Load the adenovirus-containing supernatant to the column

5. Load 15 mL of supernatant to the **AV Maxi Column** and let the supernatant gradually run through the column. Transfer the flow through to another clean tube. Keep loading until all samples pass through the column.

   Optional: Reload the flow through to ensure 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 min, take off the **Press-On Cap**, and put the column into 50 mL Centrifugal Tube. Centrifuge at 4℃, 1,000 x g for 2-5 min. Transfer the flow through to another clean tube if reloading is needed. Keep loading the supernatant till all samples pass through the column.

IV. Wash the column and elute the adenovirus

6. Wash the **AV Maxi Column** with **10 mL 1 x AV Wash Buffer**. Repeat once. This step can be performed either by gravity flow or centrifugation at 4℃, 1,000 x g for 5 min.

7. Elute the virus by applying **4 mL 1 x AV Elution Buffer**. Collect 4 mL flow though.

V. Desalting and buffer exchange

8. Apply up to 4 mL of the sample collected from step **7** to the reservoir of a **Centrifugal Filter** and centrifuge at 3,000 rpm (4℃) for 10-15 min until approximately 500 µL remains in the reservoir. Discard the flow through and add 3.5 mL PBS to the **Centrifugal Filter** and centrifuge at 4℃, 3,000 rpm for 10-15 min until approximately 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.

Note: If not using the Centrifugal Filter, 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 small increments of time and check the liquid level, repeat centrifuge to get to the expected volume.

● Typical concentration volume Vs. spin time (Swing bucket rotor, 3,000 rpm at 4℃, 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 at 4℃, 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

9. Aliquot and store the purified virus at -80℃. 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.

VI. Regeneration of the column

10. Upon completion of the purification, add **5 mL** of **Regeneration Buffer** to the column by gravity flow and then add **10 mL** of **1 x AV Wash Buffer**. Press on the cap to the bottom. Wrap the column with parafilm in a zip block bag and store at 4℃.

Trouble Shooting Guide

Problems	Solutions
Slow flow rate caused by air bubbles below the bottom filter disc	1. Fill the column to the very top with degassed water, stretch parafilm over the top of the column, making sure that there's no air trapped between the top of the liquid and the parafilm. 2. Place a thumb over the sealed column top and invert the column until the bubble is in the exit tip. 3. With the thumb, apply pressure gently to the "diaphragm" created by the parafilm until the trapped air is expelled from the tip.
Slow flow rate caused by air bubbles in the resin bed	1. Cap the column bottom and add degassed water so that the resin is covered by a height of 1-2 cm of solution. 2. Stir the resin with a clean spatula or pasteur pipette, until all portions of the resin are loosely suspended in the solution. 3. With the bottom cap on, let the column stand for 5 min until the resin settles.
Slow flow rate caused by invisible bubbles	1. With the bottom cap on, add degassed water to the resin with a height of 1-2 cm of the solution. 2. Place the entire bottom-capped column in a 50 mL Centrifugal Tube and centrifuge at 1,000 x g for 5 min at 4°C.
Supernatant very viscous	Filter the supernatant through a 0.45 μm filter unit as mentioned above in the protocol.
Cell line did not survive after infection of the purified virus	1. Dialyze the purified virus to PBS or desired buffer before infecting cell lines. 2. Use a desalting column to exchange buffer instead of dialysis.

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 BEIWO'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 BEIWO. BEIWO's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of BEIWO, to replace the products, BEIWO 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 or visit our website.

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