Ver:1904

BW-V1160 ViraTrap™ Adenovirus Purification Miniprep Kit

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Kit Contents

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AV Mini Columns	1	5	10
Press-On Cap	2	5	10
Centrifugal Filters*	2	10	20
15 mL Centrifugal Tubes	2	10	20
10 x AV Wash Buffer	5 mL	25 mL	50 mL
2 x AV Elution Buffer	5 mL	25 mL	50 mL
Regeneration Buffer	15 mL	75 mL	150 mL
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**: Centrifugal Filters (Cat# BW-CF01) can be purchased from Biomiga separately.*

Introduction

The ViraTrap™ Adenovirus Purification Miniprep 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 1-2 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.

Storage and Stability

The guaranteed shelf life is 12 months from the date of purchase. AV Mini Columns should be stored at 4℃. Store all other components 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 1-2 T75 flask or equivalent per column)

1. For a T75 flask, transfer 8 mL of supernatant to a clean **15 mL Centrifugal 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 Centrifugal Tube**. Freeze and thaw the cell lysate between 37℃ and dry ice/ethanol for three times. Combine the cell lysate with the 8 mL supernatant.

2. Centrifuge the sample at 4℃, 3,000 rpm for 10 min. 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℃.

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**.

3. Spin an **AV Mini Column** with the **15 mL Centrifugal Tube** in a swing bucket rotor at 4℃, 500 x g for 2 min. Hold the **AV Mini Column** with a clamp or other holders. Twist off the **AV Mini Column**'s bottom closure and loosen the cap, let the liquid drop by gravity flow. Equilibrate the **AV Mini Column** with 2 mL ddH_2O and then **5 mL 1x AV Wash Buffer**.

Note: Centrifugation can help remove 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 15 mL Centrifugal Tube and centrifuge at 500 x g for 5 min.

Note: There's 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 AV-containing supernatant to the columns

4. Load 5 mL supernatant to the **AV Mini Column** and let the supernatant gradually run through the **AV Mini Column**. Keep loading until all samples pass through the **AV Mini 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 min in a shaker platform. Take off the **Press-On Cap** and put the AV Mini Column into the 15 mL Centrifugal Tube. Centrifuge at 4℃, 1,000 x g for 2 min. Transfer the flow through to another clean tube if reloading is needed. Keep loading the supernatant until all samples pass through the column.

IV. Wash the column and elute the adenovirus

5. Wash the **AV Mini Column** with **5 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.

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

V. 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℃) 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.

● 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

8. 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

9. Upon completion of the purification, add **5 mL Regeneration Buffer** to the column by gravity flow and then add **5 mL 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℃.

Trouble Shooting Guide

Problems	Solutions
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 15 mL Centrifugal Tube and centrifuge at 1,000 x g for 10 min at 4℃.
Supernatant very viscous	Filter the supernatant through a 0.45 μm filter unit as mentioned above 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 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 400-115-2855 or visit our website at www.biomiga.com.cn