Ver:1912

ViraTrap™ AAV Purification Miniprep Kit (Serotype 2)

（BW-V1169）

Contents

Kit Contents

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Preps	2	10	20
AAV Mini Columns	1	5	10
Press-On Cap	1	5	10
Centrifugal Filters*	2	10	20
15 mL Centrifugal Tubes	2	10	20
AAV Binding Buffer	45 mL	225 mL	450 mL
AAV Elution Buffer	12 mL	60 mL	120 mL
Regeneration Buffer	12 mL	60 mL	120 mL
100 × Nuclease Reaction Buffer	100 µL	500 µL	1,000 µL
Nuclease (25 U/µL)	12 µL	55 µL	110 µL
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**Centrifugal Filters (Cat# BW-CF01) can be purchased from BEIWO separately.*

Introduction

Adenovirus associate viruses (AAV), belong to the replication deficient parvovirus family, are small single-stranded DNA viruses. AAVs are important gene delivery tools, which have been used in gene therapy and RNAi delivery.

Traditionally AAVs are purified by ultracentrifugation using CsCl to separate the virus particles from cellular proteins and media components. The CsCl ultracentrifugation procedure is time consuming and limited to the volume of cell lysate to be processed.

The ViraTrap™ AAV Purification Miniprep Kit is designed for efficient purification of recombinant AAV2 from rAAV vector transfected cell line. The recovery rate is 40%-60%.

Each column can be regenerated for purifying the same AAV. 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. AAV Mini Columns should be stored at 4℃. Store Nuclease, 100 × Nuclease Reaction Buffer at -20℃, and all other components at room temperature (15-25℃).

Before Starting

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

Safety Information

The adenovirus associate virus 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

✪ ddH_2O.

✪ PBS.

✪ 0.45 μm and 0.22 μm syringe filter.

✪ Rack holder for columns.

Protocol

I. Prepare AAV-infected cell lysate (For 1- 2 T75 flasks per column)

1. For adherent transfected cells, use a pasteur pipette to remove the culture medium and harvest cells with 3-5 mL PBS using a cell scraper, transfer the cells to a **15 mL Centrifugal Tube**.

2. Pellet the cells at 3,000 rpm for 10 min at 4℃. Proceed immediately to the following steps (or cell pellet can be stored at -80℃ if needed).

3. Resuspend the cell pellet in **3 mL AAV Binding Buffer.** Make sure there are no cell clumps remaining after resuspension. This is critical for the release of viral particles.

4. Add **30 µL 100 × Nuclease Reaction Buffer** and **5 µL Nuclease**, mix well by pipeting, and incubate the mixture at 37℃ for 60 min with a gentle rocking motion.

5. Collect the supernatant with rAAV from the crude by centrifugation at 4℃ at 1,000 × g for 10 min. Further clarify the supernatant by passing it through a 0.45 µm syringe filter.

II. Equilibrate the column

6. Spin an **AAV Mini Column** with the **15 mL Centrifugal Tube** in a swing bucket rotor at 4℃ at 500 × g 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 2 mL ddH_2O and then **5 mL AAV Binding Buffer**.

   Note: Centrifugation removes the bubbles created during shipping.
   Note: A swing-bucket rotor is preferred for centrifugation.
   Note: There's a **Press-On Cap** supplied in the kit for the column tip to stop the flow.
   Note: If the flow-through gets too slow, the other alternative is to set the column in a 15 mL Centrifugal Tube and centrifuge at 4℃ at 500 × g for 2 min.
   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 AAV-containing supernatant the column

7. Load the supernatant to the **AAV Mini Column** and let the lysate gradually run through the column. Collect the flow through and reload to the same column one more time to ensure maximal viral particle binding.

   Note: If the gravity flow through rate gets noticeably slower during loading or reloading of the lysate, set the column in a 15 mL Centrifugal Tube and centrifuge at 4℃ at 1,000 × g for 2 min.

IV. Wash off the nonspecific bindings and elute the AAV

8. Wash the **AAV Mini Column** with **5 mL AAV Binding Buffer**. Repeat once. This step can be performed either by gravity flow or centrifugation at 4℃ at 1,000 × g for 5 min.

9. Elute the AAV by applying **4 mL AAV Elution Buffer**. Collect 4 mL of flow through.

V. Desalting and buffer exchange

10. Apply up to 4 mL of the sample collected from step **9** to the reservoir of a **Centrifugal Filter** and centrifuge at 4℃ at 3,000 rpm for 5 min. Process the remaining sample (if any) and centrifuge until approximately 500 µL remains in the reservoir. Add 3.5 mL of PBS or any desired buffer to the reservoir and centrifuge at 4℃ at 3,000 rpm for 10-15 min until approximately 500 µL remains in the reservoir. Pipet the sample up and down several times in reservoir and transfer the virus containing solution to a clean vial. The purified virus is ready for downstream applications.

    Note: A swing bucket rotor is preferred. Fixed angle rotor requires higher speed of 7,000 rpm for 15-20 min.

    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 less time and check the liquid level, repeat centrifuge to get to the expected volume.

- Typical concentration volume vs. spin time (Swing bucket rotor, 4℃, 3,000 rpm, 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, 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

11. Aliquot and store the final 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 syringe filter before infection.

VI. Regeneration of the column

12. Upon completion of the purification, add **5 mL** of **Regeneration Buffer** to the column by gravity flow and then add **5 mL** of **AAV Binding 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. Fill the column to the very top with degassed water, stretch parafilm over the top of the column, making sure that there is 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 invisible bubbles	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.
Supernatant very viscous	Filter the supernatant through a 0.45 um filter unit as mentioned above in the protocol.
Cell line did not survive after infection of the purified virus	1. Add the Nuclease and 100 × Nuclease Reaction Buffer as mentioned in protocol. Make sure the enzyme and reaction buffer are added. 2. Alternatively, the lysate can be processed with three freeze/thaw cycles by dry ice-ethanol and 37°C water bath to reduce the viscosity and release the viral particles.

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.

Contact Us: 400-115-2855

www.beiwobiomedical.com

Customer Support:

market@beiwobiomedical.com

Technical Support:

tech@beiwobiomedical.com