VER:1912

ViraTrap™ AAV Purification Maxiprep Kit (Serotype 2)

（BW-V1269）

Contents

Kit Contents

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Centrifugal Filters*	2	4	10
50 mL Centrifugal Tubes	1	2	5
AAV Binding Buffer	100 mL	200 mL	500 mL
AAV Elution Buffer	15 mL	30 mL	75 mL
Regeneration Buffer	40 mL	80 mL	200 mL
100 × Nuclease Reaction Buffer	250 µL	500 µL	1,250 µL
Nuclease (25 U/µL)	32 µL	65 µL	160 µL
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**Centrifugal Filters (Cat# BW-CF01) can be purchased from BEIWO separately.*

Introduction

Adeno-assiciated viruses, which 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 Maxiprep Kit is designed for the efficient purification of rAAV (Serotype 2) vector transfected cell line. The recovery rate is 40%-60%.

Each column can be regenerated for purifying the same rAAV. 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 Maxi 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 5-6 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 per flask using a cell scraper. Combine the cells into a **50 mL Centrifugal Tube**.
2. Pellet the cells at 4℃ at 3,000 rpm for 10 min. Cell pellet can be stored at -80℃ (for later use) or proceed immediately to the following step.
3. Resuspend the cell pellet in **10 mL AAV Binding Buffer**. Make sure there are no cell clumps remain after resuspension. This is critical for the release of viral particles.
4. Add **100 µL 100 × Nuclease Reaction Buffer** and **15 µL Nuclease**, mix well by pipeting and incubate the mixture at 37℃ for 30-60 min with gentle rocking motion.
5. Collect the supernatant with rAAV from the crude by centrifugation at 4℃ at 3,000 rpm for 15 min. Further clarify the supernatant by passing it through a 0.45 µm syringe filter.

II. Equilibrate the column

6. Spin the **AAV Maxi Column** with the **50 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 4 mL of ddH_2O and then **8 mL AAV Binding Buffer.**

   Note: Centrifugation removes the bubbles created during shipping.
   Note: 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℃ at 500 × g for 2 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 AAV-containing lysate to the column

7. Load the supernatant to the **AAV Maxi 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 the loading or reloading of the lysate, set the column in a 50 mL conical tube and centrifuge at 4℃ at 1,000 × g for 2 min. Repeat two times to ensure maximal viral particle binding.

   Note: The visible and invisible bubbles in the resin bed created during shipping or loading the buffers and lysate will normally cause a slow flow rate.

IV. Wash off the nonspecific bindings and elute the AAVs

8. Wash the **AAV Maxi Column** with **10 mL AAV Binding Buffer.** Repeat once. This step can be performed either by gravity flow or centrifugation at 1,000 × g for 2 min.

9. Elute the AAV by applying **4 mL AAV Elution Buffer**. Collect 4 mL 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 3,000 rpm for 5 min at 4℃. Process the remaining sample (if any) and centrifuge till approximately 500 µL remains in the reservoir. Add 3.5 mL of PBS or any desired buffer to the reservoir and centrifuge at 3,000 rpm for 10-15 min at 4℃ until approximately 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. 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. See “Typical concentration volume Vs. spin time on page **6**”.

    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

  Optional: Sterilize the purified virus by passing it through a 0.22 µm syringe filter. The filter unit retains some virus particles after filtration, elute the filter unit with 300 µL of desired low salt buffer to collect the retained virus particles.

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 **8 mL Regeneration Buffer** to the column by gravity flow and then add **10 mL 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. 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 conical tube and centrifuge at 1,000 × g for 5 min at 4℃.
Supernatant very viscous	Filter the supernatant through a 0.45 μm filter unit.
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 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 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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