Ver:1905

ViraTrap™ AAV Purification Miniprep Kit (All serotypes)
（BW-V1369）

Contents

Kit Contents

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Preps	2	10	20
AAV Mini Columns	1	5	10
Press-On Cap	2	10	20
Centrifugal Filters*	2	10	20
15 mL Centrifugal Tubes	1	5	10
Nuclease (25 U/μL)	11 μL	55 μL	110 μL
100 x Nuclease Reaction Buffer	100 μL	500 μL	1,000 μL
Buffer VB	8 mL	40 mL	80 mL
Buffer VP	3 mL	15 mL	60 mL
Buffer VS	20 mL	100 mL	200 mL
Buffer ES	10 mL	50 mL	100 mL
Regeneration Buffer	12 mL	60 mL	120 mL
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**: Centrifugal Filters (Cat# BW-CF01) can be purchased from BEIWO separately.*

Introduction

The ViraTrap™ AAV Purification Miniprep Kit is designed for fast and efficient purification of recombinant AAV from AAV transfected cell culture supernatant. Up to 40-60% viral particles can be purified from cell culture media of 1-2 T75 flasks.

Traditionally, the recombinant AAV 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 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, Buffer VS and Buffer ES should be stored at 4℃. Store Nuclease and 100 x Nuclease Reaction Buffer at -20℃, and 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 AAV 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. PBS.
2. 0.45 μm and 0.22 μm filters.
3. Rack holder for columns.

Protocol

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

1. For AAV transfected cells, use a pasteur pipette to remove the culture medium and harvest cells with 3-5 mL PBS. Pellet the cells at 4℃, 3,000 rpm for 10 min. Discard supernatant. Resuspend the cells in **3 mL Buffer VB**. Make sure there's no cell clumps remaining after resuspension.

2. Add **30 µL 100 x Nuclease Reaction Buffer** and **5 µL Nuclease**. Mix well by pipetting and incubate at 37℃ for 30 min. Centrifuge at 4℃, 1,000 x g for 15 min, transfer the supernatant to a clean tube, and further clarify the supernatant through a 0.45 µm filter unit. Add **1 volume Buffer VP** to **3 volume** of virus lysate (For example, add 1 mL of Buffer VP to 3 mL of virus lysate). Mix well and incubate at 4℃ overnight. The virus is stable in **Buffer VP**.

3. Centrifuge the sample at 4℃, 3,000 rpm for 30 min (Proceed to step **4** during centrifugation) . Carefully aspirate the supernatant. Spin briefly and remove the residual supernatant. The virus containing pellet should be visible. The pellet may appear hazy. Keep the virus on ice and proceed to step **5**.

II. Purification column preparation

4. Inverting an **AAV Mini Column** to resuspend the resin inside the column. Put the column into a **15 mL Centrifugal Tube** and centrifuge at 4℃, 500 × g for 2 min. Tear off the breakoff tip on the bottom of the column and place the column into the **15 mL Centrifugal Tube**. Loosen the cap to allow buffer drain out from the column by gravity. Once the liquid stops dripping, add **4 mL Buffer VS** evenly to the column and let it drain out by gravity without drying the column out.

   Note: A **Press-On Cap** for the bottom tip of the column is provided for stopping the gravity flow at any time.

5. Dissolve the pellet from step **3** with **4 mL Buffer VS** by pipetting and vortexing. Spin the sample at 3,000 rpm for 10 min at 4℃ and transfer the clear supernatant to a clean tube.

6. Load the sample from step **5** to the reservoir of a **Centrifugal Filter** and centrifuge at 4℃, 3,000 rpm for 15-20 min until around 300 µL of sample remains in the reservoir. Transfer the sample to a clean vial. Wash the reservoir by **100 µL Buffer VS** and transfer the sample to the

clean vial.

III. Load the sample to the purification column

7. Apply the sample from step **6** evenly to the **AAV Mini Column** and let it flow into the resin by gravity. Once the sample gets into the resin, proceed to next step.

Note: Slowly add the sample dropwise to the resin. Once the entire sample gets into the resin, proceed to next step. Do not let the column dry out.

IV. Elute AAV from the purification column

8. Add **4 mL Buffer ES** evenly to the column and collect 4 mL of the flow-through. The virus is in the flow through liquid.

V. Concentration

9. Apply 4 mL of the sample to the reservoir of a **Centrifugal Filter** and centrifuge at 4℃, 3,000 rpm for 10-20 min until approximately 500 µL remains in the reservoir. Pipet the solution up and down several times in the reservoir and transfer the virus containing solution to a clean vial.

Note: A swing bucket rotor is preferred. Fixed angle rotor requires higher speed of 7,000 rpm at 4℃ 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 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, 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

10. The purified virus is ready for downstream applications. 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 sterile filter before infection.

VI. Regeneration of the column

Upon completion of the purification, add **5 mL Regeneration Buffer** to the column and let the buffer passes through the column by gravity flow. Wash the column by 10 mL of PBS, let the PBS pass through the column by gravity flow. Once the liquid stops dripping, fill the column with 2 mL of PBS. Press on the cap to the bottom. Screw on the cap and 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	Cap the bottom of the column with the press on cap and spin the column at 4℃, 1,000 x g for 5 min.
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 conical tube and centrifuge at 4℃, 1,000 x g for 10 min.
Supernatant very viscous	Filter the supernatant through a 0.45 µm filter unit as mentioned in steps above.
Column clogged after loading sample	Resuspend and dissolve the virus pellet completely with Buffer VS. Spin down briefly to remove any insoluble debris.

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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