AAV maxi purification kit, all serotypes

Contents

V140608

Introduction

The ViraTrap™ AAV purification maxi kit is designed for fast and efficient purification of all rAAV serotypres from AAV infected cell culture. Viral particles can be purified from cell culture of 5 to 6 T75 flasks per column. The viruses are first applied to a purification column and then further purified, concentrated through a centrifugal filter.

Before Starting

Familiar with each step by reading this menu and prepare all materials for the procedure. Follow safety regulations for AAV.

Kit Components

Catalog#	V1469-00	V1469-01	V1469-02	Storage
Preps	1	4	10	
AAV Columns	1	2	5	4°C
Press-On Cap	1	4	10	RT
Centrifugal Filters	2	8	10	RT
Nuclease	25 µL	100 µL	250 µL	-20°C
100x Nuclease Buffer	150 µL	500 µL	1250 µL	-20°C
Buffer VB	15 mL	50 mL	125 mL	RT
Buffer VP	10 mL	40 mL	100 mL	RT
Buffer VS	15 mL	50 mL	125 mL	RT
Buffer ES	10 mL	40 mL	100 mL	RT
Regeneration Buffer	10 mL	25 mL	55 mL	RT

Stability

The Guaranteed shelf life is 12 months from the date of purchase. Store AAV columns at 4°C. Store Nuclease and 100xNuclease Buffer at-20°C, and all other components at RT.

Safety considerations

The AAV infected cell culture and the purified virus can be bio-hazardous material and can be infectious to human and animals. All protocols MUST be performed under Bio-Safety working conditions for AAV according to regulations.

Materials required but not supplied

- Standard TC centrifuge
- Swing bucket rotor.
- 0.45 µm filter unit
- Rack holder for column

Harvest AAV infected cells (cells from 4 x T75 flasks per column)

1. For adherent transfected cells, use a pasteur pipette to remove the culture medium and harvest cells using a cell scraper with 3-5 mL PBS per T75 flask. Pellet the cells at 1000 rpm for 10 min at 4°C. Discard supernatant. Resuspend the cells in 3 mL of Buffer VB. Make sure there's no cell clumps remaining after resuspension.

2. For each 3 mL of sample, add 30 µL of 100x Nuclease reaction buffer and 5 µL of Nuclease. Mix well by pipetting and incubate at 37°C for 30 minutes. Centrifuge at 1000 rpm for 15 min at 4°C, transfer the supernatant to a clean tube, further clarify the supernatant through a 0.45 µM filter unit. Add 1 volume of 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°C for at least 4 hours to overnight. The virus is stable in Buffer VP.

3. Centrifuge the sample at 6,000 rpm for 30 minutes (Proceed to step 3 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 "4".

Purification column preparation

4. Inverting an AAV column to resuspend the resin inside the column. Put the column into a 50 mL conical tube and centrifuge at 600xg for 2 minutes. Tear off the breakoff tip on the bottom of the column and place the column into the 50 mL tube. Loosen the cap to allow buffer drain out from the column by gravity.

   Once the liquid stops dripping, add 4 mL of 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 of Buffer VS by pipetting and vortexing briefly. Spin the sample at 3000 rpm for 5 min at 4°C and transfer the clear supernatant to a clean tube. Repeat spinning at 3,000 rpm at 4°C for 5 minutes and transfer the clear lysate to a clean tube.

6. Load the sample from step “5” to the reservoir of a centrifugal filter and centrifuge at 3000 rpm for 15-20 minutes till around 500 µL of sample remains in the reservoir. Transfer the sample to a clean vial. Wash the reservoir by 50 µL of Buffer VS and transfer the sample to the clean vial.

Load the sample to the purification column

7. Apply the sample from step “6” evenly to an AAV column and let it flow into the resin by gravity. Once the sample gets into the resin, proceed to the next step.

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

Elute AAV from the purification column

8. Add 6 mL of Buffer ES evenly to the column and collect 6 mL of the flow-through. The virus is in the flow through liquid.

Concentration

9. Apply 4 mL of the sample to the reservoir of a centrifugal filter and centrifuge at 3,000 rpm for 10 min at 4°C, add the remaining sample to the reservoir and centrifuge at 3,000 rpm for 15-30 min at 4°C till approximately 1000 µL sample 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 7000 rpm for 15-20 minutes.
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. Avoid over spin.

10. The purified virus is ready for downstream applications. Aliquot and store the purified virus at -80°C. 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 0.2 µm sterile filter (low protein binding) before infection.

- Typical concentration volume Vs. spin time (Swing bucket rotor, 3,000 rpm at RT, 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 , 7000 rpm at RT, 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

Regeneration of the column

11. Upon completion of the purification, add 10 mL of Regeneration Buffer to the column and let the buffer passes through the column by gravity flow. Wash the column by 2x10 mL of PBS**,** let the PBS pass through the column by gravity flow. Once the liquid stops dripping, fill the column with 10 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°C.

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 600 x g for 5 minutes.
Slow flow rate caused by invisible bubbles	• With the bottom cap on, add degassed water to the resin with a height of 1-2 cm of the solution. • Place the entire bottom-capped column in a 50 mL conical tube and centrifuge at 10 minutes at 400 x g.
Supernatant very viscous	• Forgot to filter the supernatant through a 0.45 μM filter unit.
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 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.

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