AAV2 Mini Purification Kit

Contents

V140304

Introduction

Adeno-assiciated viruses, 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 mini purification kit is designed for efficient purification of recombinant AAV2 and AAV-DJ from rAAV vector transfected cell line. Up to 1 x 10^{12} viral particles can be purified from cell lysate of 2 x T75 flasks.

Each column can be regenerated for purifying the same rAAV. For optimized viral binding and recovery, each column can be regenerated only once.

Before starting

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

Kit Components

Catalog#	V1169-00	V1169-01	V1169-02	Notes
Preps	2	10	20	
AAV Mini Columns	1	5	10	(Store at 4°C)
Press-On Cap	1	5	10	Store at RT
15 mL Collection Tube	2	10	20	Store at RT
AAV Binding Buffer	30 mL	200 mL	400 mL	Store at RT
AAV Elution Buffer	10 mL	50 mL	100 mL	Store at RT
Regeneration Buffer	10 mL	30 mL	100 mL	Store at RT
100xNuclease Reaction Buffer	100 µL	500 µL	1000 µL	Store at 4ºC
Nuclease (25 u/µL)	12 µL	55 µL	110 µL	Store at -20°C
Centrifugal Filter*	1	5	10	Store at RT

*****_Centrifugal Filter can be purchased from Biomiga separately._

Stability

The Guaranteed shelf life is 12 months from the date of purchase.

Safety considerations

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 required but not supplied

- ddH_2O
- PBS
- 0.45 µm filter unit and 0.22 µm syringe filter
- Rack holder for column

Prepare AAV-infected cell lysate (For up to 2 x 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.
2. Pellet the cells at 350 x g for 10 minutes. Cell pellet can be stored at -80°C or proceed immediately to the following step.
3. Resuspend the cell pellet in 3 mL Binding Buffer. Make sure there's no cell clumps remain after resuspension. This is critical for the release of viral particles.
4. Add 30 µL of 100 x Nuclease Reaction Buffer and 5 µL of Nuclease and incubate the mixture at 37°C for 60 minutes with gentle rocking.
5. Collect the supernatant with rAAV from the crude by centrifugation at 600 x g for 10 minutes. Further clarify the supernatant by passing through a 0.45 µm sterile syringe filter.

Equilibrate the column

6. Set the column in a 15 mL centrifuge tube and spin at 500 x g for 2 minutes. 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 of ddH_2O and then 5 mL Binding Buffer.

- Centrifugation removes the bubbles created during shipping.
- A swing-bucket rotor is preferred for centrifugation.
- If the flow-through gets too slow, the other alternative is to set the column in a 50 mL conical tube and centrifuge at 500 x g for 1 minute.
- There's a press-on cap supplied in the kit for the column tip to stop the flow.
- If the flow-through is too slow, make sure to remove any visible bubbles (See trouble shooting on page 7).

Load the AAV-containing supernatant to the column

7. Load the supernatant to the 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 slow during loading or reloading of the lysate, set the column in a 15 mL conical tube and centrifuge at 300 x g for 2 minutes.

Wash off the nonspecific bindings and elute the AAV

8. Wash the column with 5 mL Binding Buffer. Repeat once. This step can be performed either by gravity flow or centrifugation at 500 x g.

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

Desalting and Buffer exchange

10. Apply 4 mL of the sample to the reservoir of a centrifugal filter and centrifuge at 3,000 rpm for 10 minutes till 1 mL sample remains in the reservoir. Add 3 mL of PBS to the reservoir and mix well by pipetting. Spin at 3,000 rpm for 10-15 minutes till 500 µL sample 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 7000 rpm for 15-20 minutes. See “Typical concentration volume Vs. spin time on page 6”.
    Note: Volume Vs spin time varies with different types of rotors. Always spin less time to monitor the remaining volume. Avoid over spin.
    Note: If not using the centrifugal device, 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.

11. Aliquot and store the final 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 before infection.

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 Binding Buffer**.** Press on the cap to the bottom. Wrap the column with parafilm in a zip block bag and store at 4℃.

- 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 (35º Fixed angle rotor , 7000 rpm 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

Trouble Shooting Guide

Problems	Solutions
Slow flow rate caused by air bubbles in the resin bed	• Cap the column bottom and add water so that the resin is covered by a height of 1-2 cm of solution • Stir the resin with a clean spatula or Pasteur pipette, until all portions of the resin are loosely suspended in the solution. • With the bottom cap on, let the column stand for 5 minutes until the resin settles.
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 15 mL conical tube and centrifuge at 5 minutes at 1,000 x g.
Supernatant very viscous	• Forgot to filter the supernatant through a 0.45 µM filter unit.
Cell line didn't survive after infection of the purified virus	• Dialyze the purified virus to PBS or desired buffer before infecting cell lines. • 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 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 (858) 603-3219 or visit our website at www.biomiga.com