Biomiga, Inc.

V1269 AAV maxi purification kit

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本製品の仕様等は改良のため，予告無しに変更することがありますので，製品購入後再度ご確認下さい。

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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 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 volume of cell lysate to be processed.

The ViraTrap™ AAV maxi purification kit is designed for efficient purification of recombinant AAVs from rAAV vector transfected cell line. Up to 3×10^{13} viral particles can be purified from cell lysate of 6 to 8 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	V1269-01	V1269-02	Notes
Preps	4	10	
Maxi Columns	2	5	Can be used twice (Stored at 4°C)
Binding Buffer	250 mL	2×300 mL	Store at RT
Elution Buffer	40 mL	60 mL	Store at RT
Regeneration Buffer	30 mL	60 mL	Store at RT
100x Nuclease Reaction Buffer	800 µL	1500 µL	Store at RT
Nuclease (25 u/µL)	65 µL	160 µL	Store at -20°C
50mL Collection Tube	4	10	Store at RT

Stability

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

Safety Considerations

The rAAV infected cell lysate 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

1. ddH_2O
2. PBS
3. Cell Scrapers
4. 0.45 µm filters

Prepare AAV-infected cell lysate (For 6-8 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 one 50 mL conical tube.
2. Pellet the cells at 350 x g for 10 minutes. Cell pellet can be stored at –80 ℃ or proceed immediately to the following step.
3. Resuspend the cell pellet in *10 mL Binding Buffer*. Make sure there's no cell clumps remain after resuspension. This is critical for the release of viral particles.
4. Add *100 µL* of *100x Nuclease Reaction Buffer* and *15 µL* of *Nuclease* and incubate the mixture at 37 ℃ for 30-60 minutes with gentle rocking.
5. Collect the supernatant with rAAV from the crude by centrifugation at 600 x g for 15 minutes. Further clarify the supernatant by passing through a *0.45 µm sterile syringe filter.*

Equilibrate the column

6. Set the column in a 50 mL centrifuge tube and spin at 400 x g for 1 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 *4 mL* of *ddH_2O* and then *8 mL Binding Buffer.*

   - Centrifugation removes the bubbles created during shipping.
   - A swing-bucket rotor is preferred for centrifugation.
   - If the flow-through is too slow, the other alternative is to set the column in a 50 mL conical tube and centrifuge at 400 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 6).

Load the AAV-Containing lysate 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 50 mL conical tube and centrifuge at 300 x g for 2 minutes. Repeat two times to ensure maximal viral particle binding

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

Wash off the nonspecific bindings and elute the AAVs

8. Wash the column with *10 mL Binding Buffer*. Repeat once. This step can be performed either by gravity flow or centrifugation at 400 x g for 5 minutes.

9. Elute the AAV by applying *4-6 mL Elution Buffer.* Collect the elution at 1 ml each in tubes. The purified virus should be dialyzed to the desired buffer for downstream application. A desalting column can also be used for buffer exchange instead of dialysis. Sterilize the purified virus by passing through a 0.22 μm syringe filter.

   NOTE: This step can be processed by centrifugation at 300 x g with 2 mL elution bufferfor 5 minutes, repeat once to collect a second elution.

10. Aliquot and store the final purified virus at -80°C.

Regeneration of the column

11. Upon completion of the purification, add *5 mL* of *Regeneration Buffer* to the column by gravity flow or centrifugation and then add *8 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°C.

Trouble shootings

Problems	Solutions
Slow flow rate caused by air bubbles below the bottom filter disc	• Fill the column to the very top with degassed water, stretch Parafilm over the top of the column, making sure that there's no air trapped between the top of the liquid and the. • Place a thumb over the sealed column top and invert the column until the bubble is in the exit tip. • 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 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 50 mL conical tube and centrifuge at 10 minutes at 1,000 x g.
Lysate very viscous	• Forgot to add the nuclease and or nuclease reaction buffer. Make sure the enzyme and reaction buffer are added. • 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.
Cell line didn't survive after the infection of the purified AAV	• Dialyze the purified virus to PBS or desired buffer before infecting cell lines. • Use a desalting column to exchange buffer instead of dialysis.

Limited Use and Warranty

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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