

# AAV Maxi Purification Kit

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## Introduction

Adeno-associated 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 purification maxi kit is designed for efficient purification of rAAV (Serotype 2) transfected cell line. Viral particles from 5-6 T75 flasks can be purified per column.

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.

## Prepare AAV-infected cell lysate (For 5-6 T75 flasks per column)

### Kit Components

Catalog#	V1269-00	V1269-01	V1269-02	Notes
Preps	2	4	10	
AAV Maxi Column	1	2	5	Stored at 4°C
AAV Binding Buffer	100 mL	200 mL	3×200 mL	Store at RT
AAV Elution Buffer	20 mL	40 mL	60 mL	Store at RT
Regeneration Buffer	15 mL	30 mL	60 mL	Store at RT
100xNuclease Reaction Buffer	500 µL	800 µL	1500 µL	Store at -20°C
Nuclease (25 u/µL)	35 µL	65 µL	160 µL	Store at -20°C
Collection Tube	2	4	10	Store at RT
Centrifugal Filter*	1	2	5	Stored at RT

\*Centrifugal filters (Cat# CF01) 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<sub>2</sub>O
- ◆ PBS
- ◆ 0.45 µm filter unit
- ◆ Rack holder for 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 1000 rpm for 10 minutes. Cell pellet can be stored at -80°C 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 °C for 60 minutes with gentle rocking.
5. Collect the supernatant with rAAV from the crude by centrifugation at 1000 rpm for 10 minutes at 4°C. Further clarify the supernatant by passing through a **0.45 µm sterile syringe filter** (low protein binding).

### Equilibrate the column

6. Resuspend the resin in the column by inverting. Set the column in a 50 mL centrifuge tube and spin at 600 x g for 2 min. Hold the column with a clamp or other holders. Twist off the bottom, loosen the cap, and let the liquid drop by gravity flow. Equilibrate the column with **4 mL** of **ddH<sub>2</sub>O** 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 gets noticeably slow, set the column in a 50 mL conical tube and centrifuge at 600 x g for 2-5 min.
  - 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 AAV-containing lysate to the column

7. Load **15 mL** of the sample from step 5 to the column and let the sample gradually run through the column. Keep loading till all samples pass through the column. Optional: Collect the flow through and reload the sample 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 600 x g for 2-5 min at 4°C.

## 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 600 x g for 5 min at 4°C.
9. Elute the AAV by applying **4 mL Elution Buffer**. Collect **4 mL** flow through.

## 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 5-10 minutes till approximately **1 mL** sample remains in the reservoir. Add 3 mL of **PBS** to the reservoir and centrifuge at 3,000 rpm for 10-15 minutes till approximately **500 -1000 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:** Alternatively, the virus can also be desalted by dialysis overnight or other desalting columns.

**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:** 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. Avoid over spin.

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 **8 mL** of **Regeneration Buffer** to the column by gravity flow and then add **10 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.

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 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	<ul style="list-style-type: none"> <li>• Cap the column bottom and add water so that the resin is covered by a height of 1-2 cm of solution</li> <li>• Stir the resin with a clean spatula or Pasteur pipette, until all portions of the resin are loosely suspended in the solution.</li> <li>• With the bottom cap on, let the column stand for 5 minutes until the resin settles.</li> </ul>
Slow flow rate caused by invisible bubbles	<ul style="list-style-type: none"> <li>• With the bottom cap on, add degassed water to the resin with a height of 1-2 cm of the solution.</li> <li>• Place the entire bottom-capped column in a 50 mL conical tube and centrifuge at 5 minutes at 1,000 x g.</li> </ul>
Supernatant very viscous	<ul style="list-style-type: none"> <li>• Forgot to filter the supernatant through a 0.45 µM filter unit.</li> </ul>
Cell line didn't survive after infection of the purified virus	<ul style="list-style-type: none"> <li>• Dialyze the purified virus to PBS or desired buffer before infecting cell lines.</li> <li>• Use desalting column and perform buffer exchange.</li> </ul>

## 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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