Adeno-associated Virus Maxi Purification Kit

(Catalog # K1303-2, -4, -10)

I. Introduction:

Adeno-associated viruses (AAVs), 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. BioVision's AAV maxi purification kit is designed for efficient purification of rAAV (Serotype 2 and DJ) vector transfected cell line. Up to 3 x 10^{12} viral particles can be purified from cell lysate of 6 to 8 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.

II. Sample Type:

For fast and efficient purification of recombinant AAV2 and AAV-DJ from rAAV vector transfected cell line.

III. Kit Contents:

	K1303-2	K1303-4	K1303-10	
	2 preparations	4 preparations	10 preparations	Part Number
AAV Maxi Columns	1	2	5	K1303-XX-1
AAV Binding Buffer	100 mL	200 mL	2 X 300 mL	K1303-XX-2
AAV Elution Buffer	20 mL	40 mL	60 mL	K1303-XX-3
Regeneration Buffer	15 mL	30 mL	60 mL	K1303-XX-4
100X Nuclease Reaction Buffer	500 µL	800 µL	1500 µL	K1303-XX-5
Nuclease (25 u/µL)	35 µL	65 µL	160 µL	K1303-XX-6
Collection Tube	2	4	10	K1303-XX-7
Centrifugal Filters	2	4	5	K1303-XX-8

IV. User Supplied Reagents and Equipment:

- ddH_2O
- PBS
- 0.45 µm and 0.22 µm filters
- Rack holder for columns

V. Shipment and Storage:

All the reagents are shipped at room temperature. Except the AV mini columns and the 100X Nuclease Reaction Buffer, which is stored at 4°C and the Nuclease (25 u/µL), which is stored at -20°C, all other components are stored at room temperature. The guaranteed shelf life is 12 months from the date of purchase. DO NOT FREEZE!

VI. Virus Purification and Concentration Protocol:

The AAV infected cell media and the purified virus can be potential biohazardous material and can be infectious to human and animals. All protocols MUST be performed under at least Bio-Safety level 2 (BSL2) working condition.

VII. Prepare AAV infected cell lysate (for up to 6-8 T75 flasks per column):

a. 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.
b. Pellet the cells at 350g for 10 min. Cell pellet can be stored at -80ºC or proceed immediately to the following steps.
c. Resuspend the cell pellet in 10 mL Binding Buffer. Make sure there is no cell clumps remain after resuspension. This is critical for the release of viral particles.
d. Add 100 µL of 100X Nuclease Reaction Buffer and 15 µL of Nuclease and incubate the mixture at 37°C for 30-60 min with gentle rocking.
e. Collect the supernatant with rAAV from the crude by centrifugation at 600g for 15 min. Further clarify the supernatant by passing through a 0.45 µm sterile syringe filter.

VIII. Equilibrate the column:

a. Set the column in a 50 mL centrifuge tube and spin at 400g for 1 min. 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.

Notes:

I. Centrifugation removes the bubbles created during shipping.
II. A swing-bucket rotor is preferred for centrifugation.
III. There's a press-on cap supplied in the kit for the column tip to stop the flow.
IV. If the flow-through gets too slow, the other alternative is to set the column in a 50 mL conical tube and centrifuge at 400g for 1 min.
V. If the flow-through is too slow, make sure to remove any visible bubbles.

IX. Load the AAV containing lysate to the column:

a. 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 300g for 2 min. 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.

X. Wash off the nonspecific bindings and elute the AAVs:

a. Wash the column with 10 mL Binding Buffer. Repeat once. This step can be performed either by gravity flow or centrifugation at 500g.
b. Elute the AAV by applying 4-5 mL Elution Buffer. Collect 4-5 mL of flow through.

XI. Desalting and Buffer exchange:

a. Apply up to 4 mL of the sample collected from above to the reservoir of a centrifugal filter and centrifuge at 3,000 rpm for 5 min. Process the remaining sample if any and centrifuge till 200 -300 µL remains in the reservoir. Add 3.5 mL of PBS or any desired buffer to the reservoir and centrifuge at 3,000 rpm for 10-15 min till 200- 300 µL remains 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 min. 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.*
b. Optional: Sterilize the purified virus by passing through a 0.22 µm syringe filter. The filter unit retains some virus particles after filtration, elute the filter unit with 300 µL of desired low salt buffer to collect the retained virus particles.
c. Aliquot and store the final purified virus at -80°C

XII. Regeneration of the column:

a. 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℃.
b. Typical concentration volume vs. spin time (Swing bucket rotor, 3,000 rpm at room temperature (RT), 4 mL starting volume) for 100K centrifugal filter device
   I. *Spin time-15 min: concentrate volume 176 µL*
   II. *Spin time-20 min: concentrate volume 76 µL*
   III. *Spin time-25 min: concentrate volume 58 µL*
c. Typical concentration volume vs. spin time (35º Fixed angle rotor, 7000 rpm RT, 4 mL starting volume) for 100K centrifugal filter device
   I. *Spin time-10 min: concentrate volume 97 µL*
   II. *Spin time-15 min: concentrate volume 54 µL*
   III. *Spin time-20 min: concentrate volume 35 µL*

XIII. Related Products:

Products/Catalog Number
Adenovirus Mini Purification Kit # K1300-10, -20
Adenovirus Maxi Purification Kit # K1301-2, -4, -10
Adeno-associated Virus Mini Purification Kit # K1302-10, -20
Adeno-associated Virus Maxi Purification Kit # K1303-2, -4, -10
Adeno-associated Virus Mini Purification Kit, all serotypes # K1304-10, -20
Adeno-associated Virus Maxi Purification Kit, all serotypes # K1311-2, -4, -10
Lentivirus Mini Purification Kit # K1305-10, -50
Lentivirus Maxi Purification Kit # K1306-2, -4, -10
Retrovirus Mini Purification Kit # K1307-10, -20
Retrovirus Maxi Purification Kit # K1308-2, -4, -10
HCV Mini Purification Kit # K1309-10, -20
HCV Maxi Purification Kit # K1310-2, -4, -10

XIV. General Troubleshooting Guide:

Problems	Solution
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 5 min at 1,000g.
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.

FOR RESEARCH USE ONLY! Not to be used on humans.