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Adeno-associated Virus Mini Purification Kit

(Cat# K1302-10, -20; Store at Multiple Temperatures)

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 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 1 to 2 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:

	K1302-10	K1302-20		
	10 preparations	20 preparations	Part Number	Storage Temp.
AAV Mini Columns	5	10	K1302-XX-1	4°C
Press-On Caps	5	10	K1302-XX-2	RT
Centrifugal Filters	5	10	K1302-XX-3	RT
15 mL Collection Tube	5	20	K1302-XX-4	RT
AAV Binding Buffer	200 mL	400 mL	K1302-XX-5	RT
AAV Elution Buffer	50 mL	100 mL	K1302-XX-6	RT
Regeneration Buffer	30 mL	100 mL	K1302-XX-7	RT
100X Nuclease Reaction Buffer	500 µL	1000 µL	K1302-XX-8	4°C
Nuclease (25 u/µL)	55 µL	110 µL	K1302-XX-9	-20°C

IV. User Supplied Reagents and Equipment:

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

V. Shipment and Storage:

All the reagents are shipped on blue ice. The AV mini columns, 100X Nuclease Reaction Buffer is stored at 4°C. The Nuclease (25 u/µL) 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 2 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 3 mL Binding Buffer. Make sure there is no cell clumps remain after resuspension. This is critical for the release of viral particles.
d. Add 30 µL of 100X Nuclease Reaction Buffer and 5 µL of Nuclease and incubate the mixture at 37°C for 60 min with gentle rocking.
e. Collect the supernatant with rAAV from the crude by centrifugation at 600g for 10 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 15 mL centrifuge tube and spin at 500g for 2 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 2 mL of ddH_2O and then 5 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 500g for 1 min.
V. If the flow-through is too slow, make sure to remove any visible bubbles (see troubleshooting guide below).

IX. Load the AAV containing supernatant to the columns:

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.*

X. Wash off the non-specific bindings and elute the AAV:

a. Wash the column with 5 mL Binding Buffer. Repeat once. This step can be performed either by gravity flow or centrifugation

at 500g.

b. Elute the AAV by applying 3-5 mL Elution Buffer. Collect 3-5 mL of flow through.

XI. Desalting and Buffer exchange:

a. Apply 4 mL of the sample collected from above to the reservoir of a centrifugal filter and centrifuge at 3,000 rpm for 10 min till 1 mL sample remains in the reservoir. Add 3 mL of PBS or any desired buffer to the reservoir and centrifuge at 3,000 rpm for 10-15 min till 500 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. Note: If not using the centrifugal device, the virus can also be desalted by dialysis or other desalting columns.*

XII. Regeneration of the column:

a. 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℃.

b. Typical concentration volume vs. spin time (Swing bucket rotor, 3,000 rpm at 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 min 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 10 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.