Adeno-associated Virus Maxi Purification Kit, all serotypes

(Catalog # MBS846527-2, -4, -10)

I. Introduction:

Adeno-associated viruses (AAVs), maxi purification kit purification kit is designed for fast and efficient purification of all rAAV serotypes from AAV infected cell culture. Viral particles can be purified from cell culture of 6 to 8 T75 flasks per column. The viruses are first applied to a purification column and then further purified and concentrated through a centrifugal filter.

II. Sample Type: For fast and efficient purification of all rAAV serotypes from AAV infected cell culture.

III. Kit Contents:

	MBS846527-2	MBS846527-4	MBS846527-10	
	2 preparations	4 preparations	10 preparations	Part Number
AAV Maxi Columns	1	2	5	MBS846527-XX-1
Press-On Caps	2	8	10	MBS846527-XX-2
Centrifugal Filters	2	4	10	MBS846527-XX-3
Nuclease (25 u/µL)	50 µL	100 µL	200 µL	MBS846527-XX-4
100X Nuclease Reaction Buffer	250 µL	500 µL	1000 µL	MBS846527-XX-5
Buffer B	25 mL	50 mL	100 mL	MBS846527-XX-6
Buffer S	50 mL	100 mL	200 mL	MBS846527-XX-7
Regeneration Buffer	5 mL	15 mL	30 mL	MBS846527-XX-8

IV. User Supplied Reagents and Equipment:

- Standard TC centrifuge
- Swing bucket rotor
- 0.45 µm filter unit
- Rack holder for column

V. Shipment and Storage:

All the reagents are shipped at room temperature. The AAV maxi columns are 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. Harvest AAV infected cells (cells from 6-8 T75 flasks or equivalent):

a. Resuspend the pelleted cells in 2 mL of Buffer B per T75. Make sure there's no cell clumps remaining after resuspension.
b. For each 4 mL of sample, add 30 µL of 100X Nuclease reaction buffer and 5 µL of Nuclease. Mix well by pipetting and incubate at 37°C for 30 min. Centrifuge at 600g for 15 min, transfer the supernatant to a clean tube, further clarify the supernatant through a 0.45 µm filter unit.
c. Load the sample to the reservoir of a centrifugal filter and centrifuge at 3000 rpm for 15-20 min till around 300 µL of sample remains in the reservoir. Transfer the sample to a clean vial. Wash the reservoir by 100 µL of Buffer S and transfer the sample to the clean vial.

VIII. Purification column preparation:

a. Inverting the AAV column to resusped the resin inside the column. Put the column into a 50 mL conical tube and centrifuge at 1000 rpm for 2 min. Tear off the breakoff tip on the bottom of the column and place the column into the 50 mL tube. Loosen the cap to allow buffer drain out from the column by gravity. Once the liquid stops dripping, add 4 mL of Buffer S evenly to the column and let it drain out by gravity without drying the column out. Note: A press on cap for the bottom tip of the column is provided for stopping the gravity flow at any time.

IX. Load the sample to the purification column:

a. Apply the sample (from above) evenly to the AAV column and let it flow into the resin by gravity. Once the sample gets into the resin, proceed to next step.

X. Elute AAV from the purification column:

a. Add 4 mL of Buffer S evenly to the column and collect 4 mL of the flow through. The virus is in the flow through liquid.

XI. Concentration:

a. Apply 4 mL of the sample to the reservoir of a centrifugal filter and centrifuge at 3,000 rpm for 10-30 min till 200 -300 µL remains in the reservoir. Pipet the solution up and down several times in the reservoir and transfer the virus containing solution to a clean vial. *Note: A swing bucket rotor is preferred. Fixed angle rotor requires higher speed of 7000 rpm for 15-20 min. Note: Time for centrifugation may vary for different type rotors. Always centrifuge less time and check the liquid level, repeat centrifuge to get to the expected volume.*
b. Aliquot and store the purified virus at -80°C. Before infecting the 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.

XII. Regeneration of the column:

a. Upon completion of the purification, add 3 mL of Regeneration Buffer to the column and let the buffer passes through the column by gravity flow. Wash the column by 6 mL of PBS, let the PBS pass through the column by gravity flow. Once the liquid stops dripping, fill the column with 2 ml of PBS. Screw on the cap and wrap the column with parafilm in a zip block bag and store at 4°C.

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
Adenovirus Maxi Purification Kit
Adeno-associated Virus Mini Purification Kit
Adeno-associated Virus Maxi Purification Kit
Adeno-associated Virus Mini Purification Kit, all serotypes
Adeno-associated Virus Maxi Purification Kit, all serotypes
Lentivirus Mini Purification Kit
Lentivirus Maxi Purification Kit
Retrovirus Mini Purification Kit
Retrovirus Maxi Purification Kit
HCV Mini Purification Kit
HCV Maxi Purification Kit

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.
Column clogged after loading sample	• Resuspend and dissolve the virus pellet completely with Buffer S. Spin down briefly to remove any insoluble debris

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