Adeno-associated Virus Maxi Purification Kit, all serotypes

(Catalog # K1311-2, -4, -10; Shipping at RT; Storage at Multiple Temperatures)

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:

	K1311-2	K1311-4	K1311-10	
	2 preparations	4 preparations	10 preparations	Part Number
AAV Maxi Columns	1	2	5	K1311-XX-1
Press-On Caps	1	4	10	K1311-XX-2
Centrifugal Filters	2	8	10	K1311-XX-3
Nuclease	25 µL	100 µL	110 µL	K1311-XX-4
100X Nuclease Reaction Buffer	250 µL	500 µL	1000 µL	K1311-XX-5
Buffer B	15 mL	50 mL	100 mL	K1311-XX-6
Buffer S	15 mL	50 mL	100 mL	K1311-XX-7
Regeneration Buffer	10 mL	15 mL	30 mL	K1311-XX-8
Buffer P	20 mL	70 mL	140 mL	K1311-XX-9
Buffer ES	10 mL	40 mL	90 mL	K1311-XX-10

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 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 5-6 T75 flasks or equivalent):

1. For adherent transfected cells, use a pasteur pipette to remove the culture medium and harvest cells with 3-5 mL PBS per T75 flask. Pellet the cells at 1000 rpm for 10 minutes. Discard supernatant. Resuspend the cells in 3 mL of Buffer B. Make sure there's no cell clumps remaining after resuspension.
2. For each 3 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 minutes. Centrifuge at 600 x g for 15 minutes, transfer the supernatant to a clean tube and further clarify the supernatant through a 0.45 µM filter unit. Add 1 volume of Buffer P to 4 volume of virus lysate (For example, add 3 mL of Buffer P to 12 mL of virus lysate). Mix well and incubate at 4°C overnight. The virus is stable in Buffer P.
3. Centrifuge the sample at 3,000 rpm for 30 minutes. Carefully aspirate the supernatant. Spin briefly and remove the residual supernatant. The virus containing pellet should be visible. The pellet may appear hazy. Keep the virus on ice and proceed to step "4".

VIII. Purification column preparation:

4. Inverting the AAV column to resuspend the resin inside the column. Put the column into a 50 mL conical tube and centrifuge at 1000 rpm for 2 minutes. 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.*
5. Dissolve the pellet from step 3 with 4 mL of Buffer S by pipetting and vortexing briefly. Spin the sample at 3000 rpm for 5 min at 4°C and transfer the clear supernatant to a clean tube. Repeat spinning at 3,000 rpm at 4°C for 5 minutes and transfer the clear lysate to a clean tube.
6. Load the sample from step 5 to the reservoir of a centrifugal filter and centrifuge at 3000 rpm for 15-20 minutes till around 500 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.

IX. Load the sample to the purification column:

7. Apply the sample from step 6 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. *Note: Slowly add the sample dropwise to the resin. Once the entire sample gets into the resin, proceed to next step. Do not let the column dry out.*

X. Elute AAV from the purification column:

8. Add 4 mL of Buffer ES evenly to the column and collect 4 mL of the flow-through. The virus is in the flow through liquid.

XI. Concentration:

9. Apply 4 mL of the sample to the reservoir of a centrifugal filter and centrifuge at 3,000 rpm for 10-30 minutes till around 500 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 minutes. 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.*
   The purified virus is ready for downstream applications. Aliquot and store the purified virus at -80°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 (35o Fixed angle rotor , 7000 rpm at 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

XII. Regeneration of the column:

10. Upon completion of the purification, add 10 mL of Regeneration Buffer to the column and let the buffer passes through the column by gravity flow. Wash the column by 2x10 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. Press on the cap to the bottom. Screw on the cap and wrap the column with parafilm in a zip block bag and store at 4°C.

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 bottom of the column with the press on cap and spin the column at 300 x g for 5 minutes.
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 in Buffer S. Spin down briefly to remove any insoluble debris

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