ab288106 – Adeno-associated Virus Mini Purification Kit

For fast and efficient purification of recombinant Adenovirus (rAV) from Adenovirus transfected cell culture supernatant.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
https://www.abcam.com/ab288106

Storage and Stability

The AV mini columns and 100X Nuclease Reaction Buffer are stored at 4°C. The Nuclease (25 u/µL) is stored at -20°C. All other components are stored at room temperature. DO NOT FREEZE!

Materials Supplied

Item	Quantity	Storage Condition
AAV Mini Columns	5	4°C
Press-On Caps	5	RT
Centrifugal Filters	5	RT
15 mL Collection Tube	5	RT
AAV Binding Buffer	200 mL	RT
AAV Elution Buffer	50 mL	RT
Regeneration Buffer	30 mL	RT
100X Nuclease Reaction Buffer	500 µL	4°C
Nuclease (25 u/µL)	55 µL	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- ddH_2O (double-distilled water)
- 0.45 µm and 0.22 µm syringe filters
- PBS
- Rack holder for columns

Assay Protocol

Virus Purification and Concentration Protocol:

Δ Note: *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.*

1. 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.

Δ Note: *Cell pellet can be stored at -80°C at this stage if not immediately proceeding with the next part of the assay.*

c) Resuspend the cell pellet in 3 mL Binding Buffer. Make sure there are no cell clumps remaining 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 crude supernatant with rAAV by centrifugation at 600g for 10 min. Further clarify the supernatant by passing through a 0.45 µm sterile syringe filter.

Column Equilibration Protocol:

1. Set the column in a 15 mL centrifuge tube and spin at 500g for 2 min. Hold the column with a clamp or suitable holder. 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.

Δ Notes:

I. Centrifugation removes the bubbles created during shipping.
II. A swing-bucket rotor is preferred for centrifugation.
III. Use the 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, remove any visible bubbles by placing the cap on the bottom of the column 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).

Loading 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.

Δ 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.*

Washing off the non-specific bindings and eluting 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.

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 until 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 until 500 µL remains in the reservoir. Pipet the sample up and down several times in the reservoir and transfer the virus containing solution to a clean vial. The purified virus is ready for downstream applications.

Δ Note: *A swing bucket rotor is preferred. Fixed angle rotor requires higher speed of 7000 rpm for 15-20 min. If not using the centrifugal device, the virus can also be desalted by dialysis or other desalting columns. Time for centrifugation may vary for different types of rotors. Always centrifuge for the minimum time and check the liquid level, repeat centrifuge to get to the expected volume.*

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 lock bag and store at 4°C.

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 (35° 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

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)