ab288090 – Adenovirus Maxi 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: http://www.abcam.com/ab288090

Storage and Stability

- All the reagents are shipped at room temperature. Except the AV maxi columns and the centrifugal filters, which are stored at 4°C, all other components are stored at room temperature.
- The guaranteed shelf life is 12 months from the date of purchase.
- DO NOT FREEZE!

Materials Supplied

Item	Quantity			Storage Condition
	2 Preparation kit	4 Preparation Kit	10 Preparation Kit	
AV Maxi Columns	1	2	5	4°C
Press-On Caps	1	4	10	RT
Centrifugal Filters	1	2	5	4°C
10X AV Wash Buffer	10 mL	40 mL	80 mL	RT
2X AV Elution Buffer	10 mL	40 mL	80 mL	RT
Regeneration Buffer	10 mL	30 mL	50 mL	RT
50 mL Collection tubes	1	2	5	RT

Materials Required, Not Supplied

- ddH_2O
- PBS
- 0.45 µm and 0.22 µm SYRINGE filters
- 50 mL conical tube
- Rack holder for columns

Protocol

The Adenovirus 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. Harvest supernatant from Adenovirus infected cells (For five to six T75 flask or equivalent per column):
    a. Centrifuge the Adenovirus infected culture media at 6000g for 10 min. Filter the supernatant through a 0.45 µm filter unit.
    ∆**Note:** *Supernatant from five to six T75 flasks (80-100 mL) can be processed per column*
    b. The supernatant is ready for purification
    ∆**Note:** *The supernatant can also be stored at -80oC for future purification*

2. Equilibrate the AV Maxi Columns:
    a. Dilute the 10X Wash Buffer with ddH2O to 1X Wash Buffer .
    b. Dilute the 2X Elution Buffer with ddH2O to 1X Elution Buffer.
    c. Set the Maxi Column in a 50 mL conical tube and spin in a swing bucket rotor at 600g 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 4 mL of ddH2O and then 10 mL 1X Wash Buffer.

∆**Notes:**

- *Centrifugation can help remove the bubbles created during shipping.*
- *A swing-bucket rotor is preferred for centrifugation.*
- *If the flow-through is too slow, the other alternative is to set the column in a 50 mL conical tube and centrifuge at 600g for 2 min.*
- *There's a press-on cap supplied in the kit for the column tip to stop the flow.*
- *If the flow-through is too slow, make sure to remove any visible bubbles.*

3. Load the AV-containing supernatant to the Maxi Column
    a. Load the supernatant to the Maxi Column and let the supernatant gradually run through the column. Collect the flow through and reload to the same column one more time to ensure maximal binding.

    ∆**Note:** *If the gravity flow through rate gets noticeably slow during loading or reloading of the supernatant, set the column in a 50 mL conical tube and centrifuge at 300g for 1 min. Note: Load 15 mL of supernatant to column each time.*

4. Wash the column and elute the AV
    a. Wash the column with 10 mL AV Wash Buffer. Repeat once. This step can be performed either by gravity flow or centrifugation at 600g for 5 min.
    b. Elute the virus by applying 4 mL AV Elution Buffer. Collect the elution in tubes at 1 mL each. Elute in a clean tube.

5. Desalting and Buffer exchange
    a. Apply up to 4 mL of the sample collected from step 4b. to the reservoir of a Centrifugal Filter and centrifuge at 600 g for 5-10 min at 4°C. Discard the flow through. Process the remaining sample if any and centrifuge till approximately 500 µL remains in the reservoir. Discard the flow through and add 3.5 mL of PBS or any desired buffer to the reservoir and centrifuge at 600g for 10-15 min till 500 µL remains in the reservoir. Pipet the solution up and down several times in reservoir and transfer the virus containing solution to a clean vial.

    ∆**Note:** *Alternatively, the supernatant and dialyzed overnight. 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. Aliquot and store the purified virus at -80°C. Before infecting 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.

6. 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 5 mL 1X Wash Buffer. Press on the cap to the bottom. Wrap the column with parafilm in a zip block bag and store at 4 °C.

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