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Adenovirus Maxi Purification Kit

(Cat# K1301-2, -4, -10; Store at Multiple Temperatures)

I. Introduction:

BioVision's Adenovirus Maxi Purification kit is designed for fast and efficient purification of recombinant Adenovirus from Adenovirus (AV) transfected cell culture supernatant. Up to $1x10^{10}$ viral particles can be purified from cell culture media of five to six T75 flasks. Traditionally, the recombinant adenovirus is purified by ultra-centrifugation using CsCl to separate the virus particles from cellular proteins and media components. The CsCl ultracentrifugation procedure is time consuming and is limited to the amount of cell lysate to be processed. Each column can be regenerated for purifying the same adenovirus. For optimized viral binding and recovery, each column can be regenerated only once.

II. Sample Type: For fast and efficient purification of recombinant Adenovirus from Adenovirus transfected cell culture supernatant.

III. Kit Contents:

Components	K1301-2	K1301-4	K1301-10	Part Number	Storage Temperature
	2 Preparations	4 Preparations	10 Preparations		
AV Maxi Columns	1	2	5	K1301-XX-1	4°C
Press-On Caps	1	4	10	K1301-XX-2	RT
Centrifugal Filters	1	2	5	K1301-XX-3	RT
10X AV Wash Buffer	10 mL	40 mL	80 mL	K1301-XX-4	RT
2X AV Elution Buffer	10 mL	40 mL	80 mL	K1301-XX-5	RT
Regeneration Buffer	10 mL	30 mL	50 mL	K1301-XX-6	RT
50 mL Conical tubes	1	2	5	K1301-XX-7	RT

IV. User Supplied Reagents and Equipment:

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

V. Shipment and Storage:

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!

VI. Virus Purification and Concentration 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.

VII. 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 -80°C for future purification.*

VIII. Equilibrate the AV Maxi Columns:

a. Dilute the **10X** Wash Buffer with ddH_2O to **1X** Wash Buffer.

b. Dilute the **2X** Elution Buffer with ddH_2O 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 ddH_2O** and then **10 mL 1X Wash Buffer**.

Notes:

I. Centrifugation can help remove the bubbles created during shipping.
II. A swing-bucket rotor is preferred for centrifugation.
III. 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.
IV. There's a press-on cap supplied in the kit for the column tip to stop the flow.
V. If the flow-through is too slow, make sure to remove any visible bubbles (see troubleshooting guide).

IX. 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.

X. 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.

XI. Desalting and Buffer exchange:

a. Apply up to 4 mL of the sample collected from step **Xb**. 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.

XII. Regeneration of the column:

Upon completion of the purification, add **8 mL of Regeneration Buffer** to the column by gravity flow and then add **5 mL Binding Buffer**. Press on the cap to the bottom. Wrap the column with parafilm in a zip block bag and store at 4℃.

- 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

- 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

XIII. Related Products:

Product Name/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.