Adenovirus Mini Purification Kit

(Cat# K1300-10, -20, Shipped at RT, Store at Multiple Temperatures)

I. Introduction:

BioVision's Adenovirus mini purification kit is designed for fast and efficient purification of recombinant Adenovirus from Adenovirus (AV) transfected cell culture supernatant. Up to 3 x10^{11} viral particles can be purified from cell culture media of 1 to 2 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 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:

	K1300-10	K1300-20		Storage Temp
	10 Preparations	20 Preparations	Part Number	
AV Mini Columns	5	10	K1300-XX-1	Store at 4°C
Press-On Caps	5	10	K1300-XX-2	Store at RT
Centrifugal Filters	5	10	K1300-XX-3	Store at RT
15 mL Collection Tube	10	10	K1300-XX-4	Store at RT
10X AV Wash Buffer	30 mL	60 mL	K1300-XX-5	Store at RT
2X AV Elution Buffer	30 mL	60 mL	K1300-XX-6	Store at RT
Regeneration Buffer	30 mL	60 mL	K1300-XX-7	Store at RT

IV. User Supplied Reagents and Equipment:

- ddH_2O
- PBS
- 0.45 µm and 0.22 µm filters
- Rack holder for columns

V. Shipment and Storage:

All the reagents are shipped at room temperature. Except the AV mini columns, 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 1-2 T75 flask or equivalent per column):

a) Centrifuge the Adenovirus infected culture media at 3,000 rpm for 10 min. Filter the supernatant through a 0.45 µm filter unit. *Note: Supernatant from one to two T75 flasks can be processed per column. Up to 3x10^{11} virus particles can be purified 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 column:

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 column in a **15 mL** centrifuge tube and spin in a swing bucket rotor at **300g** 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 **2 mL** of ddH_2O and then **5 mL** 1X Wash Buffer.

Note: Centrifugation can help remove the bubbles created during shipping.

I. A swing-bucket rotor is preferred for centrifugation.

II. If the flow-through is too slow, the other alternative is to set the column in a 15 mL conical tube and centrifuge at 400g for 5 min.

III. There's a press-on cap supplied in the kit for the column tip to stop the flow.

IV. If the flow-through is too slow, make sure to remove any visible bubbles (see troubleshooting guide below).

IX. Load the AV containing supernatant to the columns:

a. Load **5 mL** of supernatant to the column and let the supernatant gradually run through the column. Transfer the flow through to another clean tube. Keep loading till all samples pass through the column. Reload the flow through to ensure maximal viral particle binding. *NOTE: If the flow rate gets noticeably slow, cap (the press-on cap to the bottom and the screw cap to the top) and invert the column to mix the supernatant and resin well. Rock the sample for 5 min in a shaker platform. Take off the press-on cap and put the column into 15 mL tube. Centrifuge at 300g for 2 min. Transfer the flow through to another clean tube if reloading is needed. Keep loading the supernatant till all samples pass through the column.*

X. Wash the column and elute the AV:

b. Wash the column with **5 mL** 1 x Wash Buffer. Repeat once. This step can be performed either by gravity flow or centrifugation at 400g for 5 min.

b. Elute the virus by applying **4 mL** Elution Buffer. Collect the elution in tubes at 1 mL each. Measure the OD_{260} and OD_{280} of each fraction to identify the virus pool.

XI. Desalting and Buffer exchange:

a. Apply up to 4 mL of the sample collected from above to the reservoir of a centrifugal filter and centrifuge at 3,000 rpm for 10-15 min till 500 µL remains in the reservoir. Add 3.5 mL of PBS or any desired buffer to the reservoir and centrifuge at

3,000 rpm for 10-15 min till 400-500 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: A swing bucket rotor is preferred.*

b. Aliquot and store the purified virus at -80°C.Before infect 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 **2.5-3.0 mL** of Regeneration Buffer to the column by gravity flow and then add **5 mL** of 1X AV Wash Buffer. Press on the cap to the bottom. Wrap the column with parafilm in a zip block bag and store at 4℃.

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 # 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.

155 S. Milpitas Blvd., Milpitas, CA 95035 USA | T: (408)493-1800 F: (408)493-1801 | www.biovision.com | tech@biovision.com