Adenovirus Purification Maxi Kit Manual

Catalog#: AV02-01, AV02-02

Bioland

For research use only

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Introduction

The Adenovirus Maxiprep Purification Kit is designed for fast and efficient purification of recombinant Adenovirus from adenovirus infected cell culture supernatant. Each column has a binding capacity of $4x10^{12}$ viral particles. Traditionally the recombinant adenovirus is purified by ultra centrifugation using CsCl to separate the virus particles from cellular proteins and media components. The CsCl ultra-centrifugation 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.

Before Starting

Familiar with each step by reading this menu and prepare all materials for the procedure.

Kit Components

Catalog#	AV02-01	AV02-02	Storage
Preps	4	10	
Maxi Columns	2	5	Store @4°C
Desalting Columns	2	5	Store at RT
50 ml Conical Tubes	4	10	Store at RT
Press-On Cap	4	10	Store at RT
10x Wash Buffer	40ml	80 ml	Store at RT
2x Elution Buffer	40 ml	80 ml	Store at RT
Regeneration Buffer	30 ml	50 ml	Store at RT

Safety considerations

The adenovirus infected cell media and the purified virus can be potential bio-hazardous material and can be infectious to human and animals. All protocols MUST be performed under at least Bio-Safety level 2 working condition.

Materials required but not supplied

- ddH_2O
- PBS
- 0.45 µm filter unit and 0.22 µm syringe filter
- Rack holder for column

Adenovirus Purification Protocol

Harvesting supernatant from Adenovirus-infected cells (up to 6xT_{75} flask or equivalent per column)

1. Centrifuge the Adenovirus-infected culture media at 3,000 rpm for 10 minutes. Filter the supernatant through a 0.45 µm filter unit. *Supernatant from six T_{75} flasks can be processed per column.*
2. The supernatant is ready for purification. *It can also be stored at -80°C for future purification.*

Equilibrate the column

Dilute the 10 x Wash Buffer with sterile ddH_2O to 1 x Wash Buffer

Dilute the 2 x Elution Buffer with sterile ddH_2O to 1 x Elution Buffer

3. Set the column in a 50 ml centrifuge tube and spin at 600 x g for 2 minutes. 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 ddH_2O** and then **10 ml 1 x Wash Buffer**.

- *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 600 x g for 1 minute.*
- *There's a press-on cap supplied in the kit for the bottom of the column to stop the flow.*
- *If the flow-through is too slow, make sure to remove any visible bubbles (See trouble shooting on page 9).*

Load column

4. Load the supernatant to the 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 viral particle binding.

- *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 300 x g for 1 minute. Repeat two times to ensure maximal viral particle binding.

- *Load 15 ml supernatant to column each time.*
- *The visible and invisible bubbles in the resin bed normally cause the slow flow rate.*

Wash the column and elute the Adenovirus

5. Wash the column with **10 ml 1xWash Buffer**. Repeat once. *This step can be performed either by gravity flow or centrifugation at 600 x g.*
6. Elute the virus by applying **4 - 6 ml 1x Elution Buffer**. Collect 4-6 ml eluate in a clean tube. Desalting and buffer change as below.

Desalting and Buffer Change

Place the desalting column in to a 15 ml conical tube. Add up to 4 ml of sample (3.5 ml if using a 23 degree fixed angle rotor) onto the filter column. Cap the conical tube.

1. Place the conical tube into the centrifuge rotor (swinging bucket preferred), counterbalance with a same weight tube.
2. Spin at 3,000rpm for approximately 10 min in a swinging bucket rotor, 7,000rpm for 20 min in a fixed angle rotor. Process the remaining sample if any and centrifuge till 200-300 µl remains in the reservoir.
3. Add 3.5 ml PBS or any desired buffer up to the 4 ml mark on the column and spin at 3,000rpm for approximately 10-15 min till 200-300 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. *Wrap the column with parafilm in a zip block bag and store at 4°C for re-use. Note: to avoid contamination, only the same virus can be used for the column.*

- Typical residual volume Vs. spin time (Swing bucket rotor, 4,000 g at RT, 4 ml starting volume) for 100K centrifugal filter device

  Spin time 10 min: concentrate volume 176 µL

  Spin time 15 min: concentrate volume 76 µL

  Spin time 20 min: concentrate volume 58 µL

- Typical Concentration Volume Vs. Spin Time (35° Fixed angle rotor 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

4. Aliquot and store the final purified virus at -80°C.
5. 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.

Regeneration of the column

Upon completion of the purification, add **8 ml 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. *Note: to avoid contamination, only the same virus can be used for the regenerated column*

Trouble shooting Guide

Problems	Solutions
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 minutes 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 50 ml conical tube and centrifuge at 10 minutes at 1,000 x g.
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.

Limited use and warranty

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