EasyPrep™ Adenovirus

Purification Mini Kit Manual

Catalog#: AV01-01, AV01-02

Bioland

For research use only

(October 2020)

Table of Contents

Introduction

The Easyprep™ Adenovirus purification mini kit is designed for fast and efficient purification of recombinant adenoviruses from adenoviral transfected cell culture supernatant. Up to $1x10^{12}$ viral particles can be purified from cell culture media of 1 to 2 T_{75} 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.

Storage and Stability

All components are guaranteed for at least 12 months from the date of purchase when stored as follows: Mini column and desalting tube should be stored at 4 °C, and all other materials at RT (22-25°C).

Before Starting

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

Kit Components

Catalog#	AV01-01	AV01-02	Storage
Preps	10	20	
AV Mini Columns	5	10	Store @4°C
Centrifugal Columns	10	20	Store at RT
15 ml Collection Tube	10	20	Store at RT
10x Wash Buffer	25 ml	50 ml	Store at RT
2x Elution Buffer	25 ml	50 ml	Store at RT
Regeneration Buffer	75 ml	150 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 (1-2 T_{75} flask or equivalent per column)

1. For a T75 flask, transfer 8 mL of supernatant to a clean 15 mL tube. Leave around 3 ml of supernatant. Collect the cells by a scraper and transfer the cells and the supernatant to a new 15 mL tube. Freeze and thaw the cell lysate between 37°C and dry ice plus ethanol for three times. Combine the cell lysate with the 8 mL supernatant.
2. Centrifuge the sample at 3,000 rpm, 4°C, for 10 minutes. Transfer and filter the supernatant through a 0.45 µM filter unit. The filtered supernatant is ready for purification. It can also be stored at -80°C.

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 AV column in a 15 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 **2 ml ddH_2O** and then **5 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 15 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 AV 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.

NOTE: If the flow rate gets noticeably slower, 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 minutes in a shaker platform. Take off the press-on cap and put the column into 15 mL tube. Centrifuge at 400 x g for 2 minutes. Transfer the flow through to another clean tube if reloading is needed. Keep loading the supernatant until all samples pass through the column.

Wash the column and elute the Adenovirus

5. Wash the column with **5 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 **3 - 5 ml 1x 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. *This step can be processed by centrifugation at 300 x g with 1 ml Elution Buffer. Repeat to collect other elutions.*

Desalting

7. Apply up to 4 mL of the sample collected from step 6 to the reservoir of a centrifugal filter and centrifuge at 3,000 rpm (4°C) for 10-15 minutes until approximately 500 µL remains in the reservoir. Discard the flow through and add 3.5 mL PBS to the reservoir and centrifuge at 3,000 rpm for 10-15 minutes until 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

Swing Bucket Rotor (3,000 rpm, 4 mL starting volume)		**35° Fixed Angle Rotor** (7000 rpm, 4 mL starting volume)	
Spin time	Volume left	Spin time	Volume left
15 min	**176 µL**	10 min	**97 µL**
20 min	**76 µL**	15 min	**54 µL**
25 min	**58 µL**	20 min	**35 µL**

8. To recover the viruses, insert a pipet tip into the bottom of the column and withdraw the sample using a side-to-side sweeping motion to ensure total recovery. The ultrafiltrate can be stored in the centrifuge tube with a cap. *For optimal recovery, remove concentrated sample immediately after centrifugation.*

9. Sterilize the purified virus by passing through a 0.22 µm syringe filter. The filter unit retains some virus particles after filtration. Elute the filter unit with 300 µL of desired low salt buffer to collect the retained virus particles.

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

Regeneration of the column

11. Upon completion of the purification, add 5 ml of **Regeneration Buffer** to the column by gravity flow and then add 5 ml of **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.

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 15 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 centrifugal column and perform buffer exchange.

Limited use and warranty

This product is intended for *in vitro* research use only. Not for use in human.

This product is warranted to perform as described in its labeling and in Bioland's literature when used in accordance with instructions. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Bioland. Bioland's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Bioland, to replace the products, Bioland shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or learn more product information, please contact us at (562) 377-2668 or visit our website at www.bioland-sci.com

Bioland Scientific LLC

14925 Paramount Blvd., Suite C

Paramount, CA 90723

USA

Tel: (877) 603-8882

Fax: (562) 733-6008

Email: service@bioland-sci.com

order@bioland-sci.com

Visit our web at **www.bioland-sci.com** and learn more about Bioland products