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Introduction

The ViraTrap™ adenovirus mini purification kit is designed for fast and efficient purification of recombinant Adenovirus from adenovirus transfected cell culture supernatant. Up to **1 x10^{12}** 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.

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 ℃, and all other materials at RT (22-25 ℃).

Before Starting

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

Kit Components

Catalog#	V1160-00	V1160-01	V1160-02	Notes
Preps	2	10	20	
Mini Columns	1	5	10	Can be used twice (**Store at 4 ℃**)
Press-On Cap	1	5	10	Store at RT
Desalting Tube*	Not Included	1	1	Can be regenarated **(Store at 4 ℃)**
15 mL Collection Tube	2	10	20	Store at RT
10 x Wash Buffer	6 mL	30 mL	60 mL	Store at RT
2 x Elution Buffer	6 mL	30 mL	60 mL	Store at RT
Regeneration Buffer	6 mL	30 mL	60 mL	Store at RT

*Desalting tube could be regenarated and applied on one kind of virus. Desalting tube could be purchased from **Biomiga**.

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

1. ddH_2O
2. PBS
3. 0.45 μm and 0.22 μm filters
4. Rack holder for columns

Protocol:

I Harvest supernatant from adenovirus-infected cells (For 1-2 T75 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**.

   Note: Supernatant from one to two T75 flasks can be processed per column. Up to 1 x10^{12} virus particles can be purified per column.

2. The supernatant is ready for purification.

   Note: The supernatant can also be stored at -80℃ for future purification.

II Equilibrate the column

Dilute the **10 x Wash Buffer** with **ddH_2O** to **1 x Wash Buffer.**

Dilute the **2 x Elution Buffer** with **ddH_2O** to **1 x Elution Buffer**.

3. Set the column in a **15 mL** centrifuge tube and spin in a swing bucket rotor at **300 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** of ddH_2O and then **5 mL 1x Wash Buffer.**

   Note:

   - 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 400 x g for 5 minutes.
   - 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 (See trouble shooting on page 6).

III Load the adenovirus-containing supernatant to the column

4. 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 minutes in a shaker platform. Take off the press-on cap and put the column into 15 mL tube. Centrifuge at 300 x g for 2 minutes. Transfer the flow through to another clean tube if reloading is needed. Keep loading the supernatant till all samples pass through the column.

IV Wash the column and elute the adenovirus

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

6. Elute the virus by applying **2-4 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. The purified virus should be **dialyzed** to the desired buffer for downstream application. The **desalting column** can also be used for buffer exchange instead of dialysis. Sterilize the purified virus by passing through **0.22 µm syringe filter.**

7. Aliquot and store the final purified virus at -80 ℃.

V Regeneration of the column

Upon completion of the purification, add **5 mL** of **Regeneration Buffer** to the column by gravity flow and then add **5 mL** of **1 x Wash Buffer**. Press on the cap to the bottom. Wrap the column with parafilm in a zip block bag and store at 4 ℃.

Trouble Shooting Guide

Problems	Solutions
Slow flow rate caused by air bubbles below the bottom filter disc	• Fill the column to the very top with degassed water, stretch Parafilm over the top of the column, making sure that there's no air trapped between the top of the liquid and the Parafilm. • Place a thumb over the sealed column top and invert the column until the bubble is in the exit tip. • With the thumb, apply pressure gently to the "diaphragm" created by the parafilm until the trapped air is expelled from the tip.
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 600 x g for 5 min.
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 a desalting column to exchange buffer instead of dialysis.

Related Products

Catalog #	Product Name	Preps	Price $
V1160-01	Adenovirus purification mini kit	10	359.00
V1160-02	Adenovirus purification mini kit	20	698.00
V1260-01	Adenovirus purification maxi kit	4	498.00
V1260-02	Adenovirus purification maxi kit	10	998.00
V1169-01	AAV purification mini kit	10	389.00
V1169-02	AAV purification mini kit	20	698.00
V1269-01	AAV purification maxi kit	4	560.00
V1269-02	AAV purification maxi kit	10	1128.00
V1170-01	Lentivirus purification mini kit	10	359.00
V1170-02	Lentivirus purification mini kit	20	698.00
V1270-01	Lentivirus purification maxi kit	4	498.00
V1270-02	Lentivirus purification maxi kit	10	998.00
V1172-01	Retrovirus purification mini kit	10	359.00
V1172-02	Retrovirus purification mini kit	20	698.00
V1272-01	Retrovirus purification maxi kit	4	498.00
V1272-02	Retrovirus purification maxi kit	10	998.00
V1176-01	HCV purification mini kit	10	389.00
V1176-02	HCV purification mini kit	20	698.00
V1276-01	HCV purification maxi kit	4	560.00
V1276-02	HCV purification maxi kit	10	1128.00

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 Biomiga'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 Biomiga. Biomiga's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Biomiga, to replace the products, Biomiga 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 (858) 603-3219 or visit our website at www.biomiga.com

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