Retrovirus Purification Miniprep Manual

Catalog# RV01-00, RV01-01, RV01-02

Bioland

For research use only

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Introduction

The Retrovirus purification miniprep kit is designed for fast and efficient purification of recombinant Retroviruses from transfected cell culture supernatant. Up to $3x10^{10}$ viral particles can be purified from cell culture media of 1 to 2 T_{75} flasks.

Traditionally the recombinant Retrovirus 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 Retrovirus. 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 columns 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#	RV01-00	RV01-01	RV01-02	Store
Preps	2	10	20	
RV Mini Columns	1	5	10	4°C
Desalting Columns	1	5	10	4°C
15 mL Collection Tube	2	10	20	RT
10x Wash Buffer	6 mL	30 mL	60 mL	RT
2x Elution Buffer	6 mL	30 mL	60 mL	RT
Regeneration Buffer	6 mL	30 mL	60 mL	RT

Safety considerations

The Retrovirus 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

Retrovirus Purification Protocol

Harvesting supernatant from Retrovirus-infected cells (1-2 T_{75} flask or equivalent per column)

1. Centrifuge the Retrovirus-infected culture media at 3,000 rpm for 10 minutes. Filter the supernatant through a 0.45 µm filter unit. *Supernatant from one to two T_{75} flasks can be processed per column. Up to 1 $x10^{12}$ virus particles can be purified per column. The supernatant 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

2. Set the 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 6).*

Load column

3. 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 15 mL conical tube and centrifuge at 300 x g for 1 minute. Repeat two times to ensure maximal viral particle binding.*
- *The visible and invisible bubbles in the resin bed normally cause the slow flow rate.*

Wash the column and elute the Retrovirus

4. 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.*
5. 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

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 4000 × g for approximately 10–20 min in a swinging bucket rotor. When using a 35 degree fixed angle rotor, spin at maximum 7500 × g for 20 min.
3. Add PBS or other desired buffer up to the 4 mL mark on the column and spin at 4000 × g for desired time (see below). The viruses are now in the residual buffer at the bottom of the column.

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

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 **1x 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 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 desalting column and perform buffer exchange.

Limited use and warranty

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

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