Ver:1905

ViraTrap™ Retrovirus Purification Miniprep Kit

（BW-V1172）

Contents

Kit Contents

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RV Mini Columns	1	5	10
Press-On Cap	2	5	10
Centrifugal Filters*	2	10	20
15 mL Centrifugal Tubes	2	10	20
10 × RV Wash Buffer	5 mL	25 mL	50 mL
2 × RV Elution Buffer	5 mL	25 mL	50 mL
Regeneration Buffer	15 mL	75 mL	150 mL
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**Centrifugal Filters（Cat# BW-CF01）can be purchased from BEIWO separately.*

Introduction

The ViraTrap™ Retrovirus Purification Miniprep Kit is designed for fast and efficient purification of recombinant retroviruses from the retrovirus-transfected cell culture supernatant. The recovery rate is 60-70%.

Traditionally, the recombinant retrovirus is purified by ultracentrifugation 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

The guaranteed shelf life is 12 months from the date of production. RV Mini Columns should be stored at 4℃. Store all other components at room temperature (15-25℃).

Before Starting

Familiarize yourself with each step by reading this user manual and prepare all of the materials for the procedure.

Safety Information

The retrovirus infected cell media and the purified virus can be potentially 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 not Supplied

✪ ddH_2O.

✪ PBS.

✪ 0.45 μm and 0.22 μm syringe filter.

✪ Rack holder for columns.

Protocol

I. Harvest supernatant from retrovirus-infected cells (1-2 T75 flask or equivalent per column)

1. Centrifuge the retrovirus-infected culture media at 3,000 rpm for 10 min at 4℃. Filter the supernatant through a 0.45 μm filter unit.

   Note: Supernatant from 1-2 T75 flasks can be processed 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 × RV Wash Buffer** with ddH_2O to **1 × RV Wash Buffer.**

Dilute the **2 × RV Elution Buffer** with ddH_2O to **1 × RV Elution Buffer.**

3. Spin the column with the **15 mL Centrifugal Tube** in a swing bucket rotor at 500 x g for 2 min at 4℃. 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 1 × RV Wash Buffer.** Centrifugation can help remove the bubbles created during shipping.

   Note: A swing-bucket rotor is preferred for centrifugation.
   Note: If the flow-through is too slow, the other alternative is to set the column in a 15 mL Centrifugal Tube and centrifuge at 500 x g for 2-5 min.
   Note: There is a **Press-On Cap** supplied in the kit for the bottom of the column to stop the flow.
   Note: If the flow-through is too slow, make sure to remove any visible bubbles (See Trouble Shooting Guide on page **7**).

III. Load the retrovirus-containing supernatant to the column

4. Load the supernatant to the **RV Mini 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 gravity flow through rate gets noticeably slower during the loading or reloading of the supernatant, set the column in a 15 mL Centrifugal Tube and centrifuge at 1,000 x g for 2-5 minutes.

IV. Wash the column and elute the retrovirus

5. Wash the **RV Mini Column** with **5 mL 1 × RV Wash Buffer.** Repeat once. This step can be performed either by gravity flow or centrifugation at 1,000 x g for 5 min.

6. Elute the virus by applying **4 mL 1 × RV Elution Buffer.** Collect 4 mL flow through.

V. Desalting and buffer exchange

7. Apply 4 mL of the sample collected from step **6** to the reservoir of a **Centrifugal Filter** and centrifuge at 3,000 rpm for 10 min at 4°C till approximately **500 µL** sample remains in the reservoir. Add 3.5 mL of PBS or any desired low salt buffer to the reservoir and centrifuge at 3,000 rpm for 10 min at 4℃ till approximately **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 type rotor is preferred.

Note: If not using the Centrifugal Filter, the virus can also be desalted by dialysis or other desalting columns.

Note: Volume Vs spin time varies with different types of rotors. Always try to spin for the least time possible by monitoring the remaining volume. Avoid over spinning.

- Typical Concentration Volume Vs. Spin Time (Swing bucket rotor, 4℃, 3,000 rpm, 4 mL starting volume) for 100K Centrifugal Filter Device.

  Spin time-10 min: concentrate volume 176 µL

  Spin time-20 min: concentrate volume 76 µL

  Spin time-25 min: concentrate volume 58 µL

- Typical Concentration Volume Vs. Spin Time (35° Fixed angle rotor, 4℃, 7,000 rpm, 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

8. Aliquot and store the final purified virus at -80℃. Before infecting target cells, we recommend adding the needed amount of purified virus to 5-10 mL culture medium of the target cells and filter through a 0.22 μm syringe filter before infection.

VI. Regeneration of the column

9. Upon completion of the purification, add **5 mL** of **Regeneration Buffer** to the column by gravity flow and then add **5 mL** of **1 × RV 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	1.Cap the column bottom and add degassed water so that the resin is covered by a height of 1-2 cm of solution. 2.Stir the resin with a clean spatula or pasteur pipette, until all portions of the resin are loosely suspended in the solution. 3.With the bottom cap on, let the column stand for 5 min until the resin settles.
Slow flow rate caused by invisible bubbles	1.With the bottom cap on, add degassed water to the resin with a height of 1-2 cm of the solution. 2.Place the entire bottom-capped column in a 15 mL Centrifugal Tube and centrifuge at 10 min at 1,000 x g.
Supernatant very viscous	Filter the supernatant through a 0.45 µm syringe filter.
Cell line did not survive after infection of the purified virus	1.Dialyze the purified virus to PBS or desired buffer before infecting cell lines. 2.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.

This product is warranted to perform as described in its labeling and in BEIWO's literature when used in accordance with instructions. No other warranties of any kind expressed or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by BEIWO. BEIWO's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of BEIWO, to replace the products, BEIWO 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 or visit our website.

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