Ver:1905

ViraTrap™ Lentivirus Purification Miniprep Kit

（BW-V1170）

Contents

Kit Contents 2
Introduction 2
Storage and Stability 2
Before Starting 3
Safety Information 3
Materials not Supplied 3
Protocol 4
Trouble Shooting Guide 6
Limited Use and Warranty ..... 错误！未定义书签。

Kit Contents

Catalog#	BW-V1170-00	BW-V1170-01	BW-V1170-02
Preps	2	10	20
LV Mini Columns	1	5	10
Press-On Cap	2	10	20
Centrifugal Filters*	2	10	20
15 mL Centrifugal Tubes	1	5	10
Buffer P	18 mL	90 mL	180 mL
Buffer S	20 mL	100 mL	200 mL
Buffer MS	10 mL	50 mL	100 mL
Regeneration Buffer	10 mL	50 mL	100 mL
User Manual	1	1	1

**Centrifugal Filters (Cat#BW-CF01)can be purchased from BEIWO separately.*

Introduction

Traditionally the recombinant lentivirus is purified by ultracentrifugation to separate the virus particles from cellular proteins and media components. The ultracentrifugation procedure is time consuming and limited to the amount of cell lysate to be processed, in addition, the ultracentrifugation also concentrates cellular debris, membrane fragments, and unwanted proteins from culture medium.

The ViraTrap™ Lentivirus Purification Miniprep Kit is designed for fast and efficient purification of recombinant lentiviruses from lentiviral-transfected cell culture supernatant. The viruses are first pelleted from viral supernatant and then further purified and concentrated through a purification column and a desalting/concentration unit.

Viral particles can be purified from cell culture of 1 to 2 T75 flasks per column in an hour, recovery ranges from 50% to 60%. The purified virus particles can be used for downstream experiments, such as infection of animal or cells.

Each column can be regenerated for purifying the same lentivirus. 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. Store Buffer S, Buffer MS and LV Mini Columns at 4℃, and all other components at room temperature (15-25℃).

Before Starting

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

Safety Information

The lentiviral infected cell culture 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 not Supplied

1. Standard TC centrifuge.
2. Swing bucket rotor.
3. 0.45 μm and 0.22 μm filter unit.
4. Rack holder for columns.
5. PBS.

Protocol

I. Harvest lentivirus-infected culture (for 1-2 T75 flasks per column)

1. Centrifuge the lentivirus-infected culture media at 4℃, 3,000 rpm for 10 min. Filter the supernatant through a 0.45 μm filter unit. Supernatant from 1-2 T75, up to 30 mL supernatant, can be processed per prep.

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

II. Precipitation of lentivirus

2. Add **1 volume** of **Buffer P** to **3 volume** of virus supernatant (For example, add 5 mL of Buffer P to 15 mL of virus supernatant). Mix well and incubate at 4℃ for at least 4 h to overnight. The virus is stable in **Buffer P**.

3. Centrifuge the sample at 4℃, 6,000 rpm for 20-30 min (Proceed to step **4** during centrifugation). Carefully aspirate the supernatant. Spin briefly and remove the residual supernatant. The virus containing pellet should be visible. The pellet may appear hazy. Keep the virus on ice and proceed to step **5**.

III. Purification column preparation

4. Inverting and shaking the **LV Mini Column** to resuspend the resin inside the column. Put the **LV Mini Column** into a **15 mL Centrifugal Tube** and centrifuge at 4℃, 500 × g for 2 min. Tear off the tip on the bottom of the column and place the column into the **15 mL Centrifugal Tube**. Loosen the cap to allow buffer drain out from the column by gravity. Once the liquid stops dripping, add **5 mL Buffer S** evenly to the column and let it drain out by gravity without drying the column out.

   Note: A **Press-On Cap** for the bottom tip of the column is provided for stopping the gravity flow at any time.

5. Resuspend the pellet from **step 3** with **4 mL Buffer S**. Dissolve the pellet by pipetting and spin the sample at 4℃, 3,000 rpm for 5 min, transfer the clear supernatant to a clean tube. Repeat spinning at 4℃, 3,000 rpm for 5 min and transfer the clear lysate to a clean tube. Keep the virus on ice. Load the clear lysate to a **Centrifugal Filter** and spin at 4℃, 3,000 rpm for 10-15 min till approximately 300 μL remains in the reservoir.

IV. Load the sample to the purification column

6. Apply the sample evenly to the **LV Mini Column** and let it pass through the column by gravity. Discard the flow-through liquid in the collection tube.

   Note: Slowly add the sample dropwise to the resin. Once the entire sample gets into the matrix, proceed to next step. Do not let the column dry out.

III. Elute lentivirus from the purification column

7. Add **3 mL** **Buffer MS** evenly to the **LV Mini Column** and collect 3 mL the flow-through. The virus is in the flow through liquid.

VI. Concentration

8. Apply the entire sample collected from step **7** to the reservoir of a centrifugal filter and centrifuge at 4℃, 3,000 rpm for 15-20 min till approximately 500 µL remains in the reservoir. Pipet the solution up and down several times in reservoir and transfer the virus containing solution a clean vial. The purified virus is ready for downstream applications.

Note: A swing bucket rotor is preferred. Fixed angle rotor requires higher speed of 7,000 rpm for 15-20 min.

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

Note: Time for centrifugation may vary for different type rotors. Always centrifuge less time and check the liquid level, repeat centrifuge to get to the expected volume.

- Typical concentration volume Vs. spin time (Swing bucket rotor, 4℃, 3,000 rpm, 4 mL starting volume) for 100K Centrifugal Filter device

  Spin time-15 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

9. Aliquot and store the final purified virus at -80℃. 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 0.22 µm sterile filter before infection.

VII. Regeneration the column

10. Upon completion of the purification, add **3 mL** **Regeneration Buffer** to the column and let the buffer passes through the column by gravity flow. Wash the column by 6 mL PBS, and let the PBS pass through the column by gravity flow. Once the liquid stops dripping, fill the column with 2 mL PBS. Press on the cap to the bottom. Screw on the cap and 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 bottom of the column with the Press-On Cap and spin the column at 4℃, 1,000 x g for 5 min.
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 conical tube and centrifuge at 4℃, 1,000 x g for 10 min.
Supernatant is very viscous	Filter the supernatant through a 0.45 μm filter unit.
Column clogged after loading sample	Resuspend and dissolve the virus pellet completely with Buffer S. Spin down briefly to remove any insoluble debris.

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.

Contact Us: 400-115-2855
www.beiwobiomedical.com
Customer Support:
market@beiwobiomedical.com
Technical Support:
tech@beiwobiomedical.com