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# Retrovirus Mini Purification Kit

**(Cat# K1307-10, -20, Shipped at RT, Store at Multiple Temperatures)**

## I. Introduction:

BioVision's Retrovirus mini purification kit is designed for fast and efficient purification of recombinant Retroviruses from Retrovirus (RV) transfected cell culture supernatant. Up to 3 x$10^6$ viral particles can be purified from cell culture media of 1 to 2 T75 flasks. 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.

## II. Sample Type:

For fast and efficient purification of recombinant retroviruses from Retrovirus transfected cell culture supernatant.

## III. Kit Contents:

| | K1307-10 | K1307-20 | | Storage Temperature |
|---|---|---|---|---|
| | 10 preparations | 20 preparations | Part Number | |
| RV Mini Columns | 5 | 10 | K1307-XX-1 | Store at 4°C |
| Press-On Caps | 5 | 10 | K1307-XX-2 | Store at RT |
| Centrifugal Filters | 5 | 10 | K1307-XX-3 | Store at RT |
| 15 mL Collection Tube | 10 | 20 | K1307-XX-4 | Store at RT |
| 10X Wash Buffer | 30 mL | 60 mL | K1307-XX-5 | Store at RT |
| 2X Elution Buffer | 30 mL | 60 mL | K1307-XX-6 | Store at RT |
| Regeneration Buffer | 30 mL | 60 mL | K1307-XX-7 | Store at RT |

## IV. User Supplied Reagents and Equipment:

- dd$H_2O$
- 0.45 µm filter unit
- Rack holder for column
- PBS

## V. Shipment and Storage:

All the reagents are shipped at RT. Except the RV Mini Columns which is stored at 4°C, all other components are stored at room temperature. The guaranteed shelf life is 12 months from the date of purchase. DO NOT FREEZE!

## VI. Retrovirus Purification Protocol:

**The Retrovirus infected cell media and the purified virus can be potential biohazardous material and can be infectious to human and animals. All protocols MUST be performed under at least Bio-Safety level 2 working condition.**

**1. Harvesting supernatant from Retrovirus infected cells (1-2 T75 flask or equivalent per column):**

a. Centrifuge the Retrovirus infected culture media at 3,000 rpm for 10 min at 4°C. 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 3 x$10^{10}$ virus particles can be purified per column.*

b. The supernatant is ready for purification. *Note: the supernatant can also be stored at -80°C for future purification.*

**2. Equilibrate the column:**

a. Dilute the 10X Wash Buffer with dd$H_2O$ to 1X Wash Buffer.

b. Dilute the 2X Elution Buffer with dd$H_2O$ to 1X Elution Buffer.

c. Set the column in a 15 mL centrifuge tube and spin at 600g for 2 min. 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 dd$H_2O$ and then 5 mL 1X Wash Buffer.

*Notes:*

*I. Centrifugation can help remove the bubbles created during shipping.*
*II. A swing-bucket rotor is preferred for centrifugation.*
*III. If the flow-through is too slow, the other alternative is to set the column in a 15 mL conical tube and centrifuge at 600g for 2-5 min.*
*IV. There's a press-on cap supplied in the kit for the bottom of the column to stop the flow.*
*V. If the flow-through is too slow, make sure to remove any visible bubbles (See trouble shooting in page 2).*

**4. Load the Retrovirus containing supernatant to the purification column:**

a. Load 5 mL of the supernatant to the column and let the supernatant gradually run through the column. Keep loading till all samples pass through the column. Optional: 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 slow during loading or reloading of the supernatant, set the column in a 15 mL conical tube and centrifuge at 600g for 2-5 min at 4°C.*

**5. Wash the column and elute the Retrovirus from the purification column:**

Wash the column with 5 mL Wash Buffer. Repeat once. This step can be performed either by gravity flow or centrifugation at 600g for 5 min at 4°C.

**6**. Elute the virus by applying 4 mL Elution Buffer. Collect 4 ml flow through.

**7. Desalting and Buffer exchange:**

a. Apply 4 mL of the sample collected from step 6 to the reservoir of a centrifugal filter and centrifuge at 3,000 rpm for 10-15 min at 4°C till 500 µL 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 10-15 min at 4°C till 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.*

Volume Vs spin time varies with different types of rotors. Always spin less time to monitor the remaining volume. Avoid over spinning.

b. Aliquot and store the final purified virus at -80°C. Before infecting 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 0.2 µm sterile filter before infection.

**8. Regeneration of the column:**

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

- Typical concentration volume vs. spin time (Swing bucket rotor, 3,000g at room temperature (RT), 4 mL starting volume) for 100K centrifugal filter device:
  I. Spin time-10 min: concentrate volume 176 µL
  II. Spin time-20 min: concentrate volume 76 µL
  III. Spin time-25 min: concentrate volume 58 µL
- Typical Concentration Volume vs. Spin Time (35° Fixed angle rotor,7000 rpm RT, 4 mL starting volume) for 100K centrifugal filter device
  I. Spin time-10 min: concentrate volume 97 µL
  II. Spin time-15 min: concentrate volume 54 µL
  III. Spin time-20 min: concentrate volume 35 µL

## VII. Related Products:

| Products/Catalog Number |
|---|
| Adenovirus Mini Purification Kit Cat. No. K1300-10, -20 |
| Adenovirus Maxi Purification Kit Cat. No. K1301-2, -4, -10 |
| Adeno-associated Virus Mini Purification Kit Cat. No. K1302-10, -20 |
| Adeno-associated Virus Maxi Purification Kit Cat. No. K1303-2, -4, -10 |
| Adeno-associated Virus Mini Purification Kit, all serotypes Cat. No. K1304-10, -20 |
| Adeno-associated Virus Maxi Purification Kit, all serotypes Cat. No. K1311-2, -4, -10 |
| Lentivirus Mini Purification Kit Cat. No. K1305-10, -50 |
| Lentivirus Maxi Purification Kit Cat. No. K1306-2, -4, -10 |
| Retrovirus Mini Purification Kit Cat. No. K1307-10, -20 |
| Retrovirus Maxi Purification Kit Cat. No. K1308-2, -4, -10 |
| HCV Mini Purification Kit Cat. No. K1309-10, -20 |
| HCV Maxi Purification Kit Cat. No. K1310-2, -4, -10 |

## VIII. General Troubleshooting Guide:

| Problems | Solution |
|---|---|
| **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<br>• Stir the resin with a clean spatula or Pasteur pipette, until all portions of the resin are loosely suspended in the solution.<br>• With the bottom cap on, let the column stand for 5 min 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.<br>• Place the entire bottom-capped column in a 15 mL collection tube and centrifuge at 10 min at 1,000g. |
| **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.<br>• Use desalting column and perform buffer exchange. |

*FOR RESEARCH USE ONLY! Not to be used on humans.*