

EXOBX® Lentivirus Purification Kit (Magnetic Beads)

Cat. no. R1201

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Cat. No.	Reagent In Kit	Size	Storage
R1201-A	Buffer LP	35 mL	4 °C
R1201-B	Buffer LS	15 mL	4 °C
R1201-C	Buffer LE	50 mL	4 °C
R1201-D	Buffer LT	3 mL	4 °C
R1201-E	Magnetic Beads	3.5 mL	4 °C
R1201-F	Syringe Filter	10	RT

1. Transportation and Storage

- This product is shipped at ambient temperature, stored at 4°C, and has a shelf life of 2 years.

2. Important Information (Please read before using the kit)

- 1) Magnetic beads must be stored at 4°C; freezing will inactivate them.
- 2) The lentivirus purification system provided in this kit is designed for 20 mL per sample. If the volume of lentivirus-containing culture supernatant is insufficient, it can be adjusted using sterile ddH₂O (filtered through a 0.2 µm membrane filter). Alternatively, the volume of reagents in the kit can be proportionally reduced to establish a smaller-scale lentivirus purification system. However, do not arbitrarily alter the reagent ratios, as this may result in purification failure. Additionally, the volume of magnetic beads used per sample must not be less than 0.1 mL, as lower amounts will compromise purification efficiency.
- 3) Purified lentivirus can be diluted with culture medium at different ratios (e.g., 1:1, 1:2, 1:3) before being added to target cells for infection.
- 4) For enhanced lentiviral infection efficiency, it is strongly recommended to use the EXOBX® Lentivirus Infection **Enhancer Kit (R1401)**.
- 5) Magnetic beads settle over time; vortex thoroughly before use to ensure uniform suspension.
- 6) Please use the syringe filter provided in the kit to filter the purified lentivirus solution. Do not use conventional filters with 0.2 µm or 0.45 µm pore sizes, as this may reduce the lentiviral titer.
- 7) Store purified lentivirus at 4 °C for up to one month or at -80 °C for long-term storage (viral activity may decrease upon freezing)
- 8) **For safety, wear a lab coat, latex gloves, and protective eyewear when handling lentivirus. Use a biosafety cabinet to prevent accidental viral exposure.**
- 9) This product is for research use only!

3. Product Introduction

The EXOBX® Lentivirus Purification Kit (Magnetic Beads) is a magnetic bead-based lentivirus purification reagent. In a specific buffer system, the magnetic beads bind to lentivirus, which can then be eluted using PBS or ddH₂O. After purification, the lentiviral titer is increased by more than 10-fold. Additionally, lentivirus eluted with PBS or ddH₂O is free of other compounds, making it suitable for direct in vivo injection.

4. Features and Advantages

- 1) High purification efficiency: Purified lentivirus titers reach 6.25×10^7 -8 TU/mL.
- 2) Purity: The purified virus is free of other compounds and is suitable for in vivo applications.
- 3) Fast and simple: The purification process can be completed within 2 hours.

5. Experimental Procedure

- 1) Lentivirus Packaging: Perform lentivirus packaging according to the EXOBX® Lentivirus Packaging Kit (R1101) manual.
- 2) Lentivirus Collection: At 48 hours post-transfection, collect 10 mL of virus-containing supernatant, add 10 mL of fresh medium, and continue culturing for another 24-48 hours before collecting a second batch.
- 3) Clarification: Centrifuge the virus-containing supernatant at 1,000 rpm (100 g), 4°C for 5 min to remove cell debris. Store all collected supernatants at 4°C (do not freeze).
- 4) Lentivirus Purification: This purification system is optimized for a 20 mL sample. Follow the recommended protocol for optimal results.

Table 1. Composition of the Lentivirus Purification System

Reagent Name	Volume (mL)	Proportion (%)
Buffer LP	3	15%
Buffer LS	1	5%
Buffer LT	0.2	1%
Magnetic Beads	0.35	1.75%
Lentivirus Supernatant	15.45	77.25%
Total	20	100%

- 5) Magnetic Bead Collection: After incubation, transfer the centrifuge tube to a centrifuge and centrifuge at 3000 g, 4°C for 5 minutes to pellet the magnetic beads. Discard the supernatant. Alternatively, place the centrifuge tube on a magnetic rack at 4°C for 5-10 minutes to allow the beads to aggregate, then discard the supernatant.
- 6) Removal of Residual Liquid: Transfer the tube containing the magnetic bead pellet to a centrifuge and centrifuge at 3000 g, 4°C for 2 minutes. Use a pipette to completely remove any remaining liquid in the tube.
- 7) Elution: Add 1 mL of Buffer LE (PBS or ddH₂O) and vortex vigorously for 1 min.
- 8) Final Centrifugation and Collection: Centrifuge at 7000 g, 4°C for 5 minutes. Transfer the tube to a magnetic rack, and carefully transfer the supernatant into a new sterile EP tube. This supernatant contains the purified lentivirus solution.

- 9) Filtration: Use the syringe filter provided in the kit to filter the purified lentivirus solution and remove impurities.
- 10) Usage and Storage: The purified lentivirus can be used immediately for titer measurement (using the Lentivirus Titer Detection Card, which provides results within 15 minutes) or for infecting target cells. Alternatively, it can be stored at 4°C for up to one month.
- 11) Lentivirus Infection: Mix the purified lentivirus solution with cell culture medium at different ratios and add it to the target cells. Add Polybrene or the EXOBX® Lentivirus Infection Enhancer (Cat: R1401) to improve infection efficiency.
- 12) Expression Analysis: 24 hours post-infection, replace the medium with fresh culture medium. 72 hours post-infection, assess GFP fluorescence intensity under a fluorescence microscope or analyze the expression of the target protein.

6. Evaluation of Lentivirus Purification Efficiency

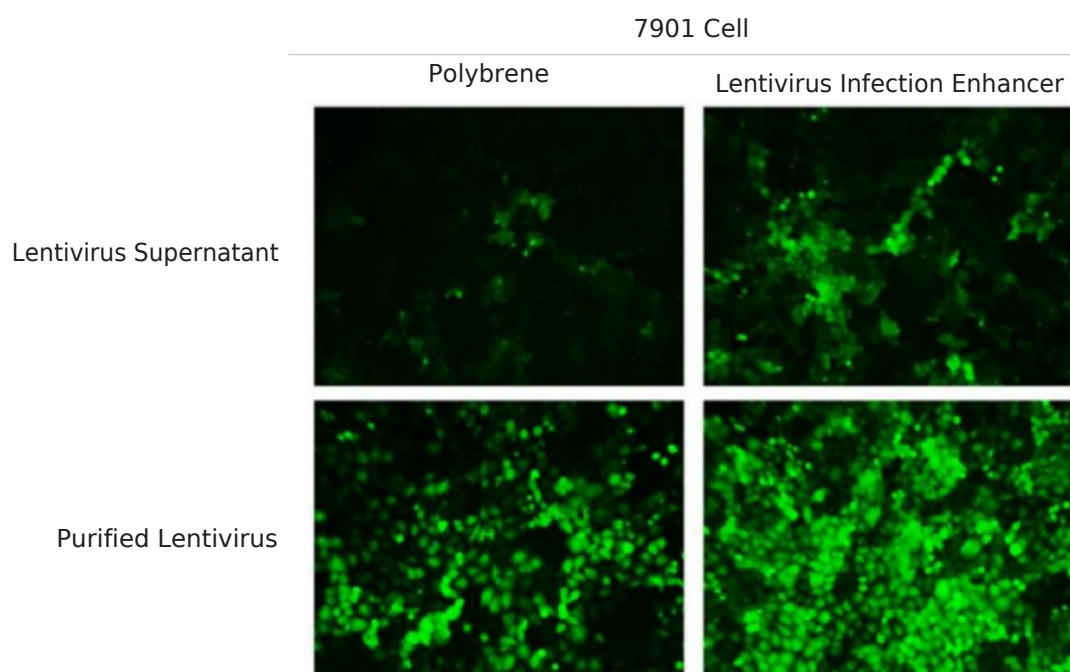


Fig. 1. Validation of lentivirus packaging, purification, and infect target cells.

293TN cells were used to package lentivirus (Lenti-GFP), which was then purified and used to infect 7901 cells. Lentivirus purification significantly enhanced its infection efficiency, and the lentivirus infection enhancer further improved the infection efficiency of both purified and unpurified lentivirus.

7. Troubleshooting

Issue	Possible Cause	Solution
Low purified lentiviral titer	Low initial viral titer in the culture supernatant.	Use a culture supernatant with a higher lentiviral titer or concentrate the sample before purification.
	Insufficient volume of virus-containing culture supernatant.	Increase the amount of virus-containing culture supernatant or scale up the purification system.
	Expired or degraded purification reagents.	Ensure that all reagents are used within their shelf life.
	Magnetic beads were frozen, reducing their ability to bind lentivirus.	Use fresh magnetic beads and store them at 4 °C without freezing.
	Incorrect reagent proportions used in the purification process.	Follow the protocol precisely and maintain the recommended reagent ratios.
	Insufficient incubation time for virus binding to magnetic beads.	Extend the incubation period to allow full virus adsorption.
	Weak elution efficiency.	Increase vortex intensity or extend vortex duration.
	Lentivirus lost during filtration due to using small-pore membrane filters.	Do not use 0.2 µm or 0.45 µm filters for purified lentivirus. Use the syringe filter provided in the kit.
	Loss of lentivirus due to high-speed centrifugation.	When centrifuging the culture supernatant, use 1000 rpm (100 g), 4 °C, for 5 minutes to remove debris without excessive viral loss.
Low infection efficiency	Insufficient amount of purified lentivirus used for infection.	Increase the volume of purified lentivirus added to target cells.
	Low Polybrene efficiency.	Use the EXOBX® Lentivirus Infection Enhancer Kit (R1401) for improved infection efficiency.
	High cell density at the time of infection.	Maintain a cell density below 70% during infection.
	Poor cell condition.	Use actively growing log-phase cells for infection.
	Repeated freeze-thaw cycles of purified lentivirus.	Use freshly purified lentivirus whenever possible; store at 4 °C for short-term use and avoid multiple freeze-thaw cycles.