

EXOBX® Lentivirus Packaging Kit

Cat. no. R1101

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Cat. No.	Reagent In Kit	Size	Storage
R1101-A	Lenti-Mix (Lentivirus Packaging Plasmid)	10 tubes	-20 °C
R1101-B	Lenti-GFP (Lentivirus Control Plasmid)	1 tube	-20 °C
R1101-C	Plasmid DNA Transfection Reagent	1 mL	4 °C
R1101-D	Polybrene (Lentivirus Infection Enhancer)	1 mL	4 °C

1. Transportation and Storage

- This product is shipped under low-temperature conditions. Each component must be stored separately according to the specified conditions. The shelf life is 2 years.
- DNA transfection reagent and Polybrene should be stored at 4 °C. Freezing is strictly prohibited.

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

- 1) Lenti-Mix and Lenti-GFP have small volumes. Before use, centrifuge at 12,000×g, 4 °C for 30 seconds to collect the plasmid solution at the bottom.
- 2) Use high-purity Lenti-shRNA plasmid or lentivirus-expressing plasmid for transfection. The OD260/OD280 ratio should be within 1.7-1.9.
- 3) The growth status of 293T(N) cells is crucial for lentivirus packaging. Cells within 20 passages are recommended.
- 4) The cell density at the time of transfection should be between 60-80%. A low density results in reduced virus production, while an excessively high density decreases transfection efficiency, leading to lower viral titers.
- 5) Plasmid DNA Transfection Reagent and Polybrene must be stored at 4 °C and should not be frozen.
- 6) To enhance lentiviral infection efficiency, the use of the EXOBX® Lentivirus Infection Enhancer Kit (R1401) is recommended. Its infection enhancement efficiency is 4-8 times higher than Polybrene.
- 7) For safety, wear a lab coat, latex gloves, and protective eyewear when handling lentivirus. Use a biosafety cabinet to prevent accidental viral exposure.**
- 8) This product is for research use only!

3. Product Introduction

The EXOBX® Lentivirus Packaging Kit contains lentivirus packaging plasmids (Lenti-Mix), a positive control plasmid (Lenti-GFP), a plasmid DNA transfection reagent, and a viral infection enhancer (Polybrene). The packaging plasmids can be co-transfected with lentiviral backbone plasmids to produce high-titer lentivirus.

4. Features and Advantages

- 1) The packaging plasmids in this kit are compatible with lentiviral backbone plasmids from multiple suppliers, enabling the production of high-titer lentivirus.
- 2) Plasmid DNA Transfection Reagent allows efficient transfection of large lentiviral plasmids, ensuring high transfection efficiency and increased viral yield.

5. Required Materials (Not Provided in Kit)

- 1) OPTI-MEM Medium (Gibco)
- 2) 293T cells, preferably 293TN cells (System Biosciences) for higher virus production

6. Experimental Procedure

1) Lentivirus Packaging

a. Cell Culture

- Passage 293T cells and seed them into a Φ 100 mm cell culture dish.
- Incubate the cells at 37°C in a 5% CO₂ incubator.
- After 24 hours, the cell density should reach 60–80%. (Excessive cell density may negatively affect **transfection efficiency.**)

b. Transfection

- Add 1 mL OPTI-MEM medium to the Lenti-Mix tube.
- Add 9 μ g of the lentiviral backbone plasmid (overexpression plasmid, RNAi plasmid, or GFP control plasmid) and mix thoroughly.
- Add 54 μ L of the transfection reagent Plasmid DNA Transfection Reagent, mix by pipetting 10 times, and incubate at room temperature for 30 minutes.
- Add the transfection mixture dropwise to the cell culture medium and gently shake to ensure even distribution.

c. Media Replacement

- 6–12 hours post-transfection, replace the medium with fresh culture medium and continue incubation.

d. Lentivirus Collection

- 48 hours post-transfection, collect the first batch of virus-containing culture supernatant (store temporarily at 4°C).
- Add 10 mL of fresh medium to the 293T cells and continue incubation for another 24–48 hours.
- Collect the second batch of virus-containing supernatant (which has a higher viral titer than the first batch).

e. Centrifugation

- Transfer the virus-containing culture supernatant to a centrifuge tube.
- Centrifuge at 3,000 rpm (870 g) at 4°C for 5 minutes.
- Discard the pellet and transfer the supernatant to a new sterile centrifuge tube.

- The supernatant now contains the crude lentivirus preparation.

f. Lentiviral Titer Measurement & Purification

- Determine the lentiviral titer using a lentivirus titer detection card or purify the virus using the Lentivirus Purification Kit (Cat: R1201).

g. Storage

- The virus-containing supernatant can be stored at 4°C for up to one month.
- For long-term storage, keep at -80°C.
- Note: One freeze-thaw cycle reduces viral infectivity by 30–50%.

2) Lentivirus Packaging

- a. Target Cell Culture: Passage the target cells and seed them into a Φ 100 mm cell culture dish. After 24 hours, the cell density should reach 30–60%.
- b. Lentivirus Infection: Discard the culture medium and add 5 mL of fresh culture medium along with 5 mL of lentivirus-containing supernatant. Add 5–20 μ L of Polybrene, diluted at a 1:500 to 1:2000 ratio in culture medium. Gently shake to mix thoroughly.
- c. Media Replacement: 12–24 hours post-infection, replace with fresh culture medium and continue incubation. If no cytotoxicity is observed, media replacement is not necessary.
- d. Detection: 72 hours post-infection, observe GFP expression under a fluorescence microscope. Alternatively, assess the expression level of the target gene using appropriate molecular analysis methods.

7. Efficiency Evaluation

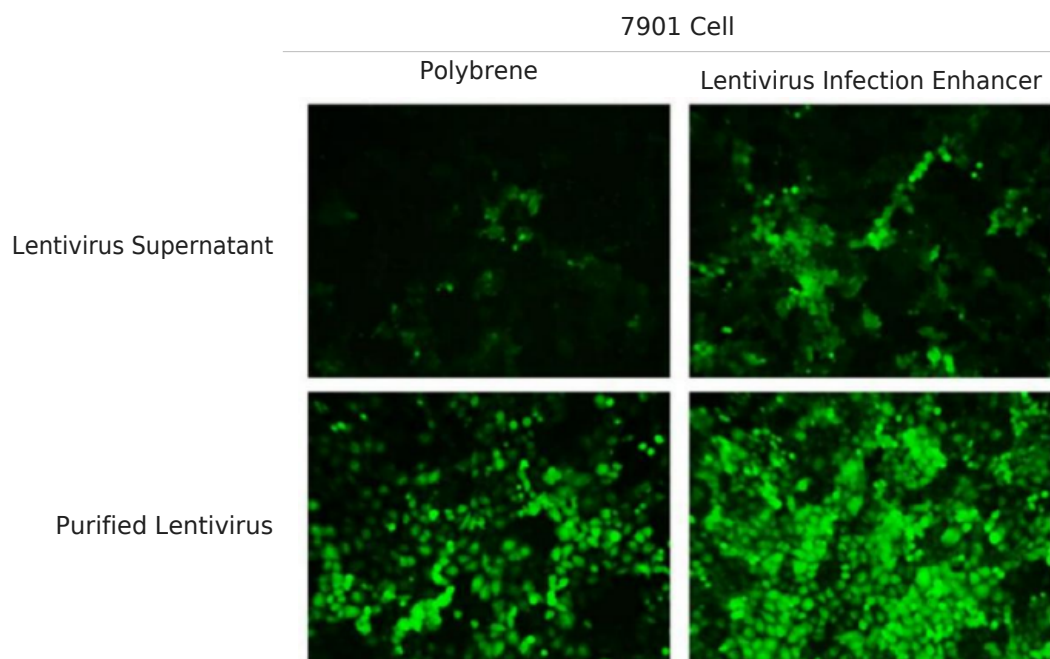


Fig. 1. Validation of lentivirus packaging, purification, and infect target cells. 293TN cells were used to package lentivirus (Lenti-GFP), which was then purified and used to infect 7901 cells. Lentivirus purification significantly enhanced its infection efficiency, and the lentivirus infection enhancer further improved the infection efficiency of both purified and unpurified lentivirus.

8. Troubleshooting

Issue	Possible Cause	Solution
Low lentiviral titer	The passage of 293T(N) cells is too high (exceeding 20 passages).	Use low-passage 293T(N) cells for lentivirus packaging.
	The cell density of 293T cells is suboptimal.	Ensure that 293T cells reach 60–80% confluency at the time of transfection.
	The purity or concentration of the lentiviral backbone plasmid is insufficient.	The OD260/OD280 ratio of the lentiviral plasmid should be 1.7–1.9, and the concentration should be at least 0.2 µg/µL.