EDITGENE Lentiviral Packaging Kit

Product Manual

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Lentiviral Packaging Kit Manual

► Product Information

Catalog Number	Specification
EDLV-L01	10T
EDLV-L02	20T

► Product Overview

The Lentiviral Packaging Kit includes optimized packaging plasmids, a control plasmid expressing GFP protein, high-efficiency transfection reagents, and lentiviral concentration reagents. This kit offers compatibility with various plasmid systems, a short packaging cycle, high viral titers, and ease of use, making it ideal for beginners in viral packaging.

► Kit Components

Component	Storage Condition	10T	20T
Lentipac-Mix Packaging Plasmid	-20 ℃	200 μL	400 μL
GFP Control Plasmid	-20 ℃	20 μL	20 μL
Trans-PEI Transfection Reagent	4 ℃	70 μL	150 μL
LentiCon-PEG Viral Concentration Solution	4 ℃	40 mL	80 mL

Note: The Lentipac-Mix Packaging Plasmid and GFP Control Plasmid stored at -20 ℃ should be protected from repeated freeze-thaw cycles. Thaw at 4 ℃ before use. If

multiple uses are expected, it is recommended to aliquot the plasmids after the initial thawing for storage.

► Additional Materials Required for Lentiviral Packaging

1. Lentiviral vector plasmid expressing the target gene.
2. Tool cells for lentiviral packaging and titer determination: HEK 293T (EDITGENE Cat No. EDC2024001).
3. Reagents for cell culture: Opti-MEM (Gibco Cat No. 31985070), PBS (Beyotime Cat No. ST476), DMEM (Gibco Cat No. C11995500BT), FBS (Gibco Cat No. FBS-S500).
4. Laboratory consumables: culture dishes, centrifuge tubes, pipette tips, pipettes, etc.

► Experimental Procedure Overview

The lentiviral vector plasmid carrying the target gene and the Lentipac-Mix packaging plasmid are co-transfected into 293T cells with the aid of Trans-PEI transfection reagent. After transfection, the cell supernatant (containing lentivirus) is filtered through a 0.45 μm PES membrane to obtain the lentivirus. Depending on the experimental requirements, the virus can be concentrated. Viral titer is then determined using fluorescence counting or qPCR. Target cells are infected with the lentivirus at an appropriate MOI based on the viral titer, followed by antibiotic or fluorescence-based selection to achieve stable cell line generation.

1. Procedure Flowchart

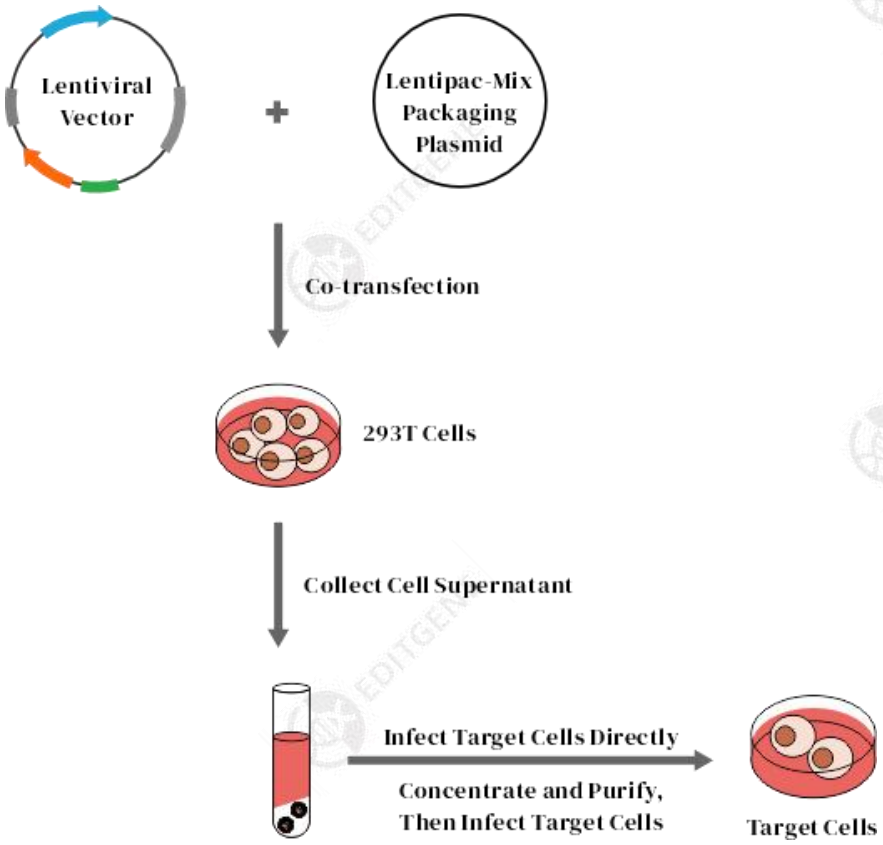


Figure 1. Schematic Diagram of the Lentiviral Packaging Process

2. Lentiviral Vector Preparation

Before starting lentiviral packaging, you need to obtain a lentiviral vector for the gene of interest. This vector may express the target gene, shRNA, or microRNA, among others. EDITGENE offers a range of lentiviral vectors, with customizable options for various promoters, selection markers, expression tags, or reporter genes to meet diverse experimental needs. Once the lentiviral vector is prepared, determine the required plasmid amount based on the desired viral packaging volume and proceed with plasmid extraction.

Note: To achieve high transfection efficiency, ensure plasmid quality. It is recommended to use a high-purity plasmid extraction kit (OMEGA Cat No. D6950-02) for plasmid preparation, with an A260/A280 ratio typically between 1.8 and 2.0.

3. Lentiviral Packaging

Co-transfect the Lentipac-Mix packaging plasmid and GFP Control Plasmid (or lentiviral vector plasmid carrying the target gene) into 293T packaging cells. The following protocol is based on a 100 mm culture dish. Adjust volumes accordingly when using other culture vessels with different surface areas.

(1) Passage 293T cells 24 hours before packaging and seed 3.6×10^6 cells in a 100 mm culture dish. Incubate at 37℃ with 5% CO_2, ensuring ~70% confluency before transfection.

(2) Prepare Solution A: Mix 20 μL Lentipac-Mix packaging plasmid, 20 μL GFP Control Plasmid (or 10 μg target gene lentiviral vector plasmid), and 80 μL Opti-MEM thoroughly.

(3) Prepare Solution B: Mix 30 μL Trans-PEI transfection reagent (3 μL per 1 μg plasmid) with 70 μL Opti-MEM thoroughly.

(4) Slowly add Solution B to Solution A while gently mixing with a pipette, and incubate at room temperature for 15-20 minutes.

(5) Replace the 293T cell medium with 12 mL of fresh complete medium.

(6) Add the 200 μL mixture to the 293T cell culture and incubate at 37℃ with 5% CO_2.

(7) After 8 hours, replace the medium with 12 mL of DMEM containing 20% FBS and continue incubating at 37℃ with 5% CO_2.

(8) After 48 hours of transfection, collect the cell supernatant and centrifuge at 800 g for 10 minutes.

(9) Using a disposable syringe, filter the supernatant through a 0.45 μm PES membrane into a 50 mL centrifuge tube.

(10) The filtered viral solution can be concentrated as needed for the experiment. If concentration is not required, aliquot the virus solution and flash-freeze with liquid nitrogen before storing at -80℃.

Note: When performing lentiviral packaging for the first time, it is recommended to conduct a preliminary experiment using the GFP Control Plasmid provided in the kit.

4. Lentiviral Concentration

(1) Add 4 mL of LentiCon-PEG Viral Concentration Solution to the viral solution. Immediately invert to mix, place on ice, and invert 5 times every 30 minutes, repeating this step 5 times. Leave the mixture overnight in a 4℃ refrigerator.

(2) Note: The white precipitate is the viral pellet. The overnight incubation step can be extended to up to one week, as a longer incubation can enhance viral titer.

(3) Pre-cool the centrifuge to 4℃, then centrifuge at 4000 g for 20 minutes.

(4) In a biosafety cabinet, carefully remove the supernatant and resuspend the viral pellet in PBS at 1/10-1/100 of the original volume.

(5) Aliquot the concentrated virus and flash-freeze with liquid nitrogen, then store at -80℃.

Note: It is recommended to use the lentivirus to transfect cells within 3 days of collection to avoid freeze-thaw cycles, which can significantly impact viral activity. If immediate use is not possible, flash-freeze in liquid nitrogen and store at -80℃.

5. Titer Determination

- **Fluorescence Counting Method**

Technical Principle: This method determines viral titer by co-culturing lentivirus with target cells and counting the number of cells expressing fluorescent proteins using a fluorescence microscope. This provides an effective measure of viral activity units (TU/mL).

(1) Plate 293T cells the day before titer measurement in a 96-well plate with 5×10^3 cells per well in 100 μL of medium.

(2) Prepare a serial dilution of the viral sample. Prepare 8 Eppendorf tubes, each containing 90 μL of complete medium. Add 10 μL of the viral sample to the first tube, mix thoroughly, then transfer 10 μL to the second tube. Repeat this process until the last tube.

(3) Label the plate wells and add 90 μL of the corresponding viral dilution to each well.

(4) Incubate at 37℃ with 5% CO_2 for 24 hours, then replace with 100 μL of fresh medium.

(5) After 48 hours, observe fluorescence expression and count fluorescent cells, or use FACS for counting. If the virus only carries puromycin resistance, add the appropriate antibiotic 48 hours post-infection and continue selection for 6-7 days, then count the resistant cells.

(6) In fields of view where the proportion of fluorescent cells is close to 50%, calculate the viral titer using the following formula:
Viral Titer (TU/mL) = Number of Fluorescent Cells (or Resistant Cells) / Viral Volume (mL)

Example: GFP-based viral titer determination

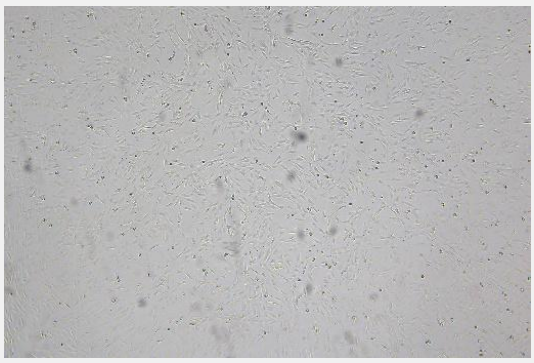
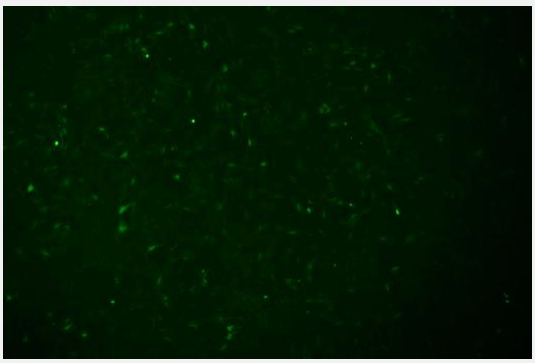
Bright Field Image	GFP Fluorescence Image
	

Figure 2. Transfection of 5 × 10^3 293T Cells with 10^{-4} mL Lentivirus, Showing Approximately 50% Fluorescent Cells

Conclusion: The viral titer is calculated as follows:

Viral Titer (TU/mL) = $5\times10^3\times50\%/10^{-4}=2.5\times10^7$ TU/mL

- **qPCR Detection Method**

Technical Principle: Specific primers are used to amplify the conserved sequence of the RNA genome in lentiviral samples based on the HIV-1 structure. The amplification products are monitored in real time via qRT-PCR. A standard curve is generated using the CT values obtained from standard samples. The CT value of the viral sample is then compared to the standard curve to calculate the RNA genome copy number in the sample, representing the viral particle count (copies/mL).

For detailed procedures, please refer to the relevant kit manual (Takara Cat No. 631235).

6. Lentiviral Infection Pre-Experiment

(1) The day before the experiment, seed 293T cells at a concentration of 3–5 × 10^4 cells/mL into 10 wells of a 96-well plate, adding 90 µL per well.

(2) Optimize lentiviral infection conditions by dividing the experiment into three groups, each with three different MOI levels (100, 10, 1):

(3) A. Complete medium with virus infection.

(4) B. Complete medium with 5 µg/mL Polybrene and virus infection.

(5) C. Complete medium with 10 µg/mL Polybrene and virus infection.

(6) Dilute the virus to the appropriate concentration based on the desired MOI and add the diluted viral solution to the corresponding wells.

(7) Incubate at 37°C with 5% CO_2 for 24 hours, then replace with fresh medium.

(8) After 3–4 days of infection, observe fluorescence expression under a fluorescence microscope. For slower-growing cells, extend the observation period and change the medium as needed to maintain cell viability.

(9) Assess the infection efficiency by observing the infected cells and confirming the optimal infection conditions and parameters for target cells. Calculate infection efficiency as follows:

Infection Efficiency = (Number of Fluorescent Cells / Total Cells in the Same Field) × 100%.

(10) If optimal infection conditions and MOI with high efficiency are not found in the pre-experiment, consider increasing the virus amount or performing antibiotic selection as necessary.

Note: The above protocol is for adherent cells. For suspension cells, seeding is not required the day before. Instead, centrifuge the cells before the experiment, resuspend them in the appropriate media, count and seed them, then add the virus.

Example: Pre-Experiment for Transfection of Human Embryonic Kidney Cells 2V6.11

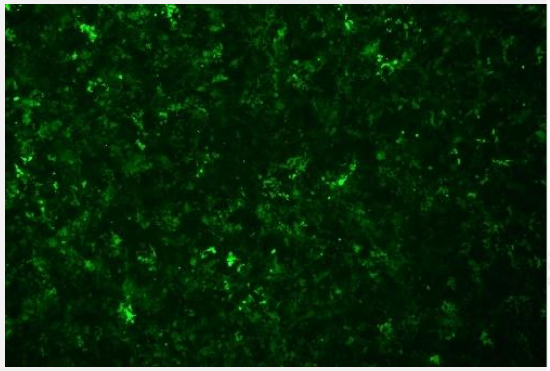
Bright Field Image	GFP Fluorescence Image
	

Figure 3. Transfection Efficiency of 2V6.11 Cells with MOI=3 and Polybrene at 10 μg/mL

Conclusion: Under the conditions of MOI=3 and 10 μg/mL Polybrene, the transfection efficiency for 2V6.11 cells is over 80%

Note: Lentivirus expression can be slow, with GFP fluorescence observable in some cells only 72-96 hours post-infection or even longer. During the later stages of infection, please monitor cell growth and perform timely media changes and passaging as needed to maintain optimal cell health.

7. Main Infection Experiment

Based on the MOI and Polybrene conditions determined in the pre-experiment, calculate the required amounts of virus and reagents. Follow the pre-experiment protocol. Refer to the table below for recommended cell numbers and culture volumes for various culture vessels:

Vessel	Surface Area (cm^2)	Recommended Cell Number	Culture Volume (mL)
35 mm	8.8	0.3×10^6	2
60 mm	21.5	0.8×10^6	5
100 mm	56.7	2.2×10^6	12
6-well	9.6	0.3×10^6	1 to 3
12-well	3.5	0.1×10^6	1 to 2
24-well	1.9	0.05×10^6	0.5 to 1.0
48-well	1.1	0.03×10^6	0.2 to 0.4
96-well	0.32	0.01×10^6	0.1 to 0.2
T-25	25	0.7×10^6	3 to 5
T-75	75	2.1×10^6	8 to 15
T-175	175	4.9×10^6	35 to 53
T-225	225	6.3×10^6	45 to 68

► Common Issues and Solutions

Low Viral Titer: This may be due to factors such as cell condition, plasmid quality, target gene size and sequence, and collection time. To improve viral titer, use high-purity, endotoxin-free plasmids, select an appropriate cell line and transfection conditions, and collect the viral supernatant at the optimal time.

Unstable Target Gene Expression: Random integration sites can lead to instability. To stabilize gene expression, consider using viral vectors with inducible expression.

Cytotoxicity: Lentiviral infection can cause cytotoxicity, affecting cell growth and survival. To reduce toxicity, select viral vectors and packaging plasmids with lower

toxicity and optimize experimental conditions to reduce the concentration of viral particles.

Immune Response: Lentiviral infection may trigger an immune response. To minimize its impact, consider adding immunosuppressive agents after viral infection.

Low Transfection Efficiency: Factors such as the choice of transfection reagent, cell condition, and plasmid quality can affect efficiency. Ensure high-quality transfection reagents and optimize conditions, such as cell density and transfection time.

Low Lentiviral Concentration Efficiency: When using concentration solutions, ensure that the mixture is kept at low temperatures to allow gradual precipitation. Room temperature can hinder precipitation, lowering concentration efficiency.

Low Viral Infectivity: This may result from issues with the viral vector or packaging plasmid quality or problems during transfection. Use high-quality viral vectors and packaging plasmids and optimize transfection conditions to improve infectivity.

Weak GFP Fluorescence Signal: This may be due to a low number of viral particles entering host cells, poor cell proliferation, cell type, or early observation time. Typically, GFP signal is strongest around 3 days post-infection.

► Precautions

(1) This product is intended for research use only in laboratory settings. Please comply with relevant laws, regulations, and ethical requirements; any consequences from improper use are the sole responsibility of the user.

(2) Follow the guidelines for transportation, storage, and use of reagents. Avoid repeated freeze-thaw cycles unless necessary. The company is not responsible for experimental failures due to improper storage or handling.

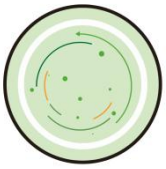
► Why Choose EDITGENE?

Simple Operation

Ready-to-use, pre-mixed packaging plasmids eliminate the need for concentration and ratio calculations, streamlining the procedure.

High Viral Yield

By utilizing a proprietary optimization of plasmid ratios and packaging processes, we effectively increase lentiviral yield.



High Compatibility

Using a second-generation lentiviral packaging system ensures safety while remaining compatible with both second- and third-generation lentiviral plasmids.

High-Titer Virus

The accompanying viral concentration solution allows for the production of higher-titer virus, making it suitable for a wider range of applications.

► Appendix

We recommend using the following vectors for modification to obtain lentiviral vectors carrying the target gene:

EDITGENE Code	Plasmid Name	Description
EDV280	lentiCRISPR-v3-PURO	CRISPR knockout vector with puromycin resistance
EDV289	lentiCRISPR-v3-BLAST	CRISPR knockout vector with blasticidin resistance
EDV1954	Lenti-CMV-MCS-EF1a-Puro	Overexpression vector with CMV promoter, no tag
EDV3021	pLV3-U6-MCS-shRNA -Cop GFP-Puro	shRNA expression vector with puromycin resistance, GFP fluorescence
EDV3022	pLV3-U6-MCS-shRNA -EF1-a-mCherry-Blast	shRNA expression vector with blasticidin resistance, mCherry fluorescence

EDITGENE offers these empty vector plasmids for purchase and provides vector modification services to streamline the lentiviral packaging process.