EurekaBio

EuLV ONE™

Lentiviral Packaging System

User Guide

For the production of lentiviral vector via transient transfection using a single plasmid

G1001
EuLV ONE™ Lentiviral Packaging Kit
(Adherent)

G1002
EuLV ONE™ Lentiviral Packaging Kit
(Suspension)

EuLV ONE™

Lentiviral Packaging System

User Guide

Revision History:
Pub. No. EU2508001

Version	Date	Description
1.0	2025.08.15	**EuLV** ONE™ Lentiviral Packaging System Product Information

Disclaimer

To the extent permitted by law, Shenzhen Eureka Biotechnology Co., Limited, and its affiliates shall not be liable for any special, incidental, indirect, punitive, multiple, or consequential damages, including but not limited to loss of profits and data loss, arising from or in connection with this document.

Important Licensing Information

These products may be subject to one or more limited-use label licenses. By using these products, you agree to the terms and conditions of all applicable limited-use label licenses.

Trademark Statement

All trademarks in this document are the property of Shenzhen Eureka Biotechnology Co., Limited, and its subsidiaries. Unauthorized use of these trademarks is prohibited.

Copyright Statement

Intellectual Property

All intellectual property rights, including patents, copyrights, and designs, contained in this document and related products are owned by Shenzhen Eureka Biotechnology Co., Limited. and its subsidiaries.

Contact Information

If you have any questions regarding the use of our products or the terms provided herein, please contact us at [+86-0755-86562586] or [eulvone.support@eurekabio.com].

Table of Contents

01 Product Information

1.1 Product Description

Unlike traditional lentiviral packaging systems that co-transfect multiple plasmids (such as packaging plasmids, envelope plasmids, and transfer plasmids), the **EuLV** ONE™ Lentiviral Packaging System allows lentiviral vector production using a single transfer plasmid. This innovation eliminates challenges related to plasmid ratio optimization and compatibility, significantly reducing operational complexity and production costs.

The **EuLV** ONE™ Packaging Cell Line combines inducible gag/pol, rev, and VSV-G genes, enabling lentiviral vector (LVV) to be produced through single plasmid transfection of the transfer construct in both adherent and suspension cultures.

The **EuLV** ONE™ Lentiviral Production System offers two optimized kits:

1. **EuLV** ONE™ Lentiviral Packaging Kit (Adherent) (Catalog No. G1001) — Used for the production of lentiviral vector using adherent cell cultures in culture dishes or cell factories.
2. **EuLV** ONE™ Lentiviral Packaging Kit (Suspension) (Catalog No. G1002) — Used for scalable lentiviral vector production in suspension cultures in test tubes, culture flasks, or bioreactors.

1.2 Contents and Descriptions

Both kits can produce 300mL of lentiviral vector.

EuLV ONE™
Lentiviral Packaging Kit (Adherent)

Catalog No. G1001

Component	Catalog No.	Amount	Storage Condition	Transport Condition
EuLV ONE™ Packaging Cell Line	G2001	1mL×3	Liquid nitrogen	Dry Ice Packs
EuLV ONE™ Lentiviral Vector Backbone Plasmid	G4001	50μL×1	-20℃	
EuLV ONE™ Lentiviral Vector EGFP Plasmid	G4002	500μL×1		
Polybrene	G50011	500μL×1		

EuLV Inducer, adherent	G50022	30mL×1	-20℃	Dry Ice Packs
Transfection Reagent	G50041	500μL×5	2℃ to 8℃ Protected from light	Ice Packs

EuLV ONE™
Lentiviral Packaging Kit (Suspension)

Catalog No. G1002

Component	Catalog No.	Amount	Storage Condition	Transport Condition
EuLV ONE™ Packaging Cell Line	G2001	1mL×3	Liquid nitrogen	Dry Ice Packs
EuLV ONE™ Lentiviral Vector Backbone Plasmid	G4001	50μL×1	-20℃	
EuLV ONE™ Lentiviral Vector EGFP Plasmid	G4002	500μL×1		
Polybrene	G50011	500μL×1		
EuLV PCL Inducer, suspension	G50051	30mL×1		
EuLV Medium I	G50031	1000mL×1	2℃ to 8℃ Protected from light	Ice Packs
Transfection Reagent	G50041	500μL×5		

1.2.1. **EuLV** ONE™ Packaging Cell Line

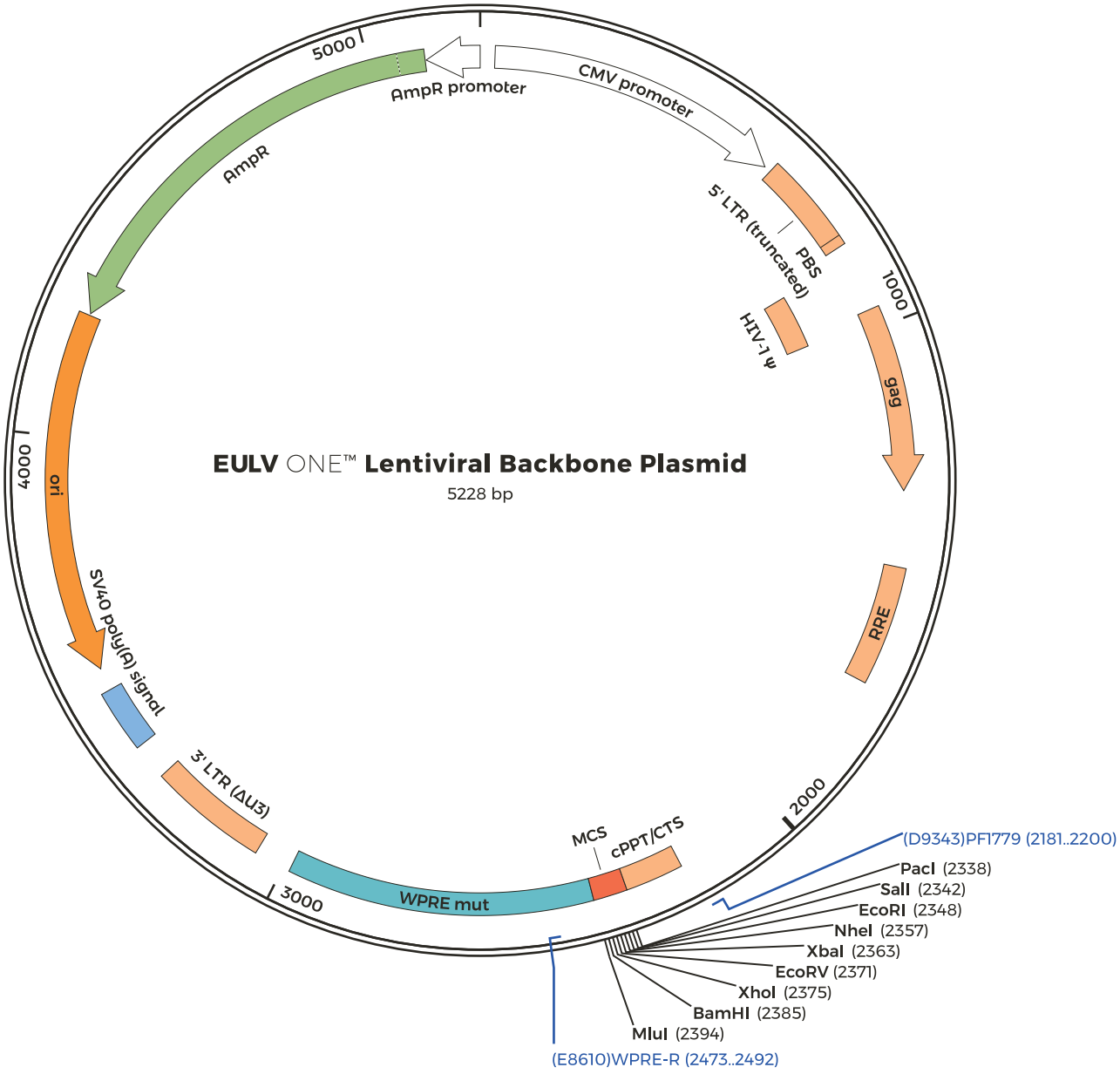
The **EuLV** ONE™ Packaging Cell Line is derived from the HEK293T cell line and has been adapted for suspension culture.

Characteristics:

- Cell doubling time of approximately 20 hours.
- High lentiviral production capacity between passage 3 and passage 20.

1.2.2. **EuLV** ONE™ Lentiviral Vector Backbone Plasmid

The **EuLV** ONE™ Lentiviral Vector Backbone Plasmid is used to construct the transfer vector plasmid required for single-plasmid transient transfection. The gene of interest (GOI) sequence can be inserted into it to produce a transfer vector plasmid.



Element Instructions

Element Name	Element Description	Element Position
CMV-RU5	CMV-RU5 chimeric regulatory element sequence regulates lentiviral RNA genome transcription	31..809
PBS	Primer binding site	811..828
HIV-1 Psi (Ψ)	Lentiviral RNA genome packaging signal element	856..981
gag	The 5′ end 1-337 bp sequence of HIV-1 gag protein coding sequence, containing 10 stop codons designed by point mutations	965..1326
RRE	rev protein response element	1480..1713
cPPT/CTS	Central polypurine tract sequence	2216..2333

MCS	Multiple cloning site	2334..2399
WPRE(mut)	BbsI restriction site mutation of woodchuck hepatitis virus post-transcriptional regulatory element	2400..2988
3′ LTR (Delta-U3)	The 3'LTR sequence of the U3 sequence was deleted, which is a lentiviral long terminal repeat sequence	3066..3299
SV40 poly(A) signal	Transcription terminator polyadenylation sequence	3371..3492
ori	Plasmid replication origin sequence	3541..4253
AmpR	Sequence encoding Ampicillin resistance gene	4260..5120
AmpR promoter	Ampicillin resistance gene promoter sequence	5121..5225

Insert the gene of interest (GOI) into the MCS site

After transfecting cells, the target protein can be transiently expressed.

1) The sequence of the MCS site is shown in the figure below.

```
   PacI     SalI   EcoRI      NheI   XbaI   EcoRV   XhoI       BamHI     MluI
ttaattaagtcgacgaattcagtgctagctctagagatatcctcgagaactggatccctaacgcgt
aattaattcagctgcttaagtcacgatcgagatctctataggagctcttgacctagggattgcgca
```

2) Recommended restriction enzyme site combinations for inserting the gene of interest (GOI):

PacI & XhoI, PacI & BamHI, PacI & MluI.

Sequencing primers used after inserting the gene of interest

1) Name and sequence of the 5′-end sequencing primer:

(D9343)PF1779: TGAACGGATCTCGACGGTAT

2) Name and sequence of the 3′-end sequencing primer:

(E8610)WPRE_R: CATTAAAGCAGCGTATCCAC

1.2.3. **EuLV** ONE™ Lentiviral Vector EGFP Plasmid

The **EuLV** ONE™ Lentiviral Vector EGFP Plasmid is a control transfer vector plasmid designed for single-plasmid transient transfection, with EGFP as the gene of interest.

1.2.4. Polybrene

Polybrene is a transduction enhancer used to improve lentiviral transduction efficiency during lentiviral titer determination.

1.2.5. EuLV Inducer, adherent

A chemically defined, animal-free, serum-free, and protein-free formulation used to initiate lentiviral vector production in adherent **EuLV** ONE™ Packaging Cell Line.

1.2.6. EuLV Medium I

The EuLV Medium I is a chemically defined, animal-free, serum-free, and protein-free formulation developed specifically for the growth and transfection of the **EuLV** ONE™ Packaging Cell Line. Prior to use, 6 mM of glutamine should be added to the medium.

> **Note**
>
> **If the medium needs to be repeatedly pre-warmed, it is recommended to aliquot it before use.**

1.2.7. Transfection Reagent

The Transfection Reagent is specially formulated for the efficient and low-toxicity transfection of transfer plasmids into the **EuLV** ONE™ Packaging Cell Line.

1.2.8. EuLV PCL Inducer, suspension

A chemically defined, animal-free, serum-free, and protein-free formulation used to initiate lentiviral vector production in suspension **EuLV** ONE™ Packaging Cell Line.

1.3. Suggested Instruments not supplied

Instrument name	Manufacturer	Model
Low speed centrifuge	Hunan Xiangyi Laboratory Instrument Development Co., Ltd	L550
CO_2 shaker incubator	Shanghai Zhichu Instrument Co., Ltd	ZCZY-AS8V, ZCZY-AS8E, ZCZY-BS8ES
Automatic cell fluorescence analyzer	Alit Biotech (Shanghai) Co., Ltd	Countstar Rigel S2
Flow cytometer	Agilent	NovoCyte 3130

1.4. Suggested Materials not supplied

Instrument name	Manufacturer	Model
Shake flask	CORNING LIFE SCIENCE (WUJIANG) CO LTD	125mL
	Guangzhou Jet Bio-Filtration Co., Ltd.	
Culture dish	CORNING LIFE SCIENCE (WUJIANG) CO LTD	100mm

02 Resuscitation, Passage, and Cryopreservation

Important Note

Store cryopreserved cells in liquid nitrogen before use.

- Avoid exposing cells to short-term extreme temperature fluctuations.
- For all cell handling procedures, gently rotate the cells to mix them and avoid vigorous shaking or pipetting.

Resuscitation

Restore frozen cells to a viable and proliferative state for experimentation, ensuring minimal damage from ice crystal formation and cytotoxic effects of cryoprotectants like dimethyl sulfoxide (DMSO).

Passage

Maintain healthy cell proliferation by passaging adherent or suspension cells at optimal confluence and cell density, preventing contact inhibition, cellular senescence, or stress induced by overgrowth.

Cryopreservation

Store cells long term at ultra-low temperatures (-196°C) using cryoprotectants (e.g., DMSO) to preserve cell viability, genetic stability, and function for future applications while minimizing the risk of contamination or genetic variation.

2.1. Adherent Cells

2.1.1. Resuscitation

a	Remove the **EuLV** ONE™ Packaging Cell Line cryovial from the liquid nitrogen and place it immediately in a 37℃ water bath, gently shaking until no visible ice remains in the cryovial. **Note: Avoid immersing the cryovial cap to prevent contamination.**
b	Transfer the cells into a 15mL centrifuge tube containing 9mL of complete DMEM medium (with 10% fetal bovine serum).
c	Centrifuge at 190×g for 3 minutes.
d	Aspirate and discard the supernatant, add 3mL of complete DMEM medium, and gently pipette to disperse the cells evenly.
e	Use a cell counter to determine the cell density and viability of live cells. Based on a concentration of 2×10^5 live cells/mL, calculate the required volume of cell suspension for seeding in a 10cm culture dish (with a culture volume of 10mL), and add medium to bring the total volume to 10mL. Label the resuspended cells as passage 1.
f	Place the culture dish in a 37℃, 5% CO_2 incubator and culture for two days.

2.1.2. Passage

a	After two days of culture, aspirate the original medium and slowly add 5mL of PBS along the inner wall of the dish. Gently swirl the dish to allow the PBS to cover the bottom, then aspirate the PBS from the dish.
b	Add 2mL of recombinant trypsin to cover the bottom of the dish. Incubate at 37℃ for 3 minutes until approximately 80% of the cells have detached (observe for rounded cell morphology under the microscope, gently tapping the dish if necessary).
c	Add 5mL of complete DMEM medium to stop the digestion, and then transfer the cell suspension to a centrifuge tube.
d	Centrifuge at 190×g for 3 minutes.
e	Aspirate and discard the supernatant, add 3mL of complete DMEM medium, and gently pipette to disperse the cells evenly.

f	Use a cell counter to determine the cell density and viability of live cells. Based on a concentration of 2×10^5 live cells/mL, calculate the required volume of cell suspension for seeding in a 10cm culture dish (with a culture volume of 10mL), and add medium to bring the total volume to 10mL. After passage, the cell passage number increases by 1. **Acceptable standard: Cell viability ≥ 80%.**
g	Place the culture dish in a 37℃, 5% CO_2 incubator for further culture and expansion.

2.1.3. Cryopreservation

a	Prepare a cryopreservation solution containing 10% DMSO by mixing fetal bovine serum, complete DMEM medium, and DMSO in a volume ratio of 5:4:1, and pre-cool it in a 2-8℃ refrigerator. **Note: The cryopreservation solution must be prepared fresh and used within 1 hour of preparation.**
b	The steps are the same as described in 2.1.2 a-2.1.2 d.
c	Aspirate and discard the supernatant. Resuspend the cells in an appropriate volume of cryopreservation solution, aliquoting 1 mL per cryovial, such that the final cell density is approximately 2×10^6 viable cells per mL. **For example: Seed 2×10^6 live cells into a 10 cm culture dish and culture for two days. When the cell count reaches 8×10^6 live cells, add 4mL of cryopreservation solution to resuspend the cells.**
d	Use a cell counter to determine the cell density and viability of live cells. **Acceptable standard: Cell viability ≥ 90%, cell density ≥ 2×10^6 live cells/mL.**
e	Aliquot 1mL of cell suspension into pre-labeled cryovials. Seal the cryovials to prevent liquid nitrogen leakage.
f	Transfer the cryovials to a pre-chilled programmable freezing container (previously stored at 2-8℃ for 30 minutes) for programmed freezing.
g	Transfer the programmed freezing container to a -80℃ ultra-low temperature freezer and store for more than 20 hours.
h	Transfer the cells to a liquid nitrogen tank for long-term storage.

2.2. Suspension Cells

2.2.1. Resuscitation

a	Remove the **EuLV** ONE™ Packaging Cell Line cryovial from the liquid nitrogen and place it immediately in a 37℃ water bath, gently shaking until no visible ice remains in the cryovial. **Note: Avoid immersing the cryovial cap to prevent contamination.**
b	Transfer the cells to a 15mL centrifuge tube containing 9mL of EuLV Medium I (with 6mM glutamine).
c	Centrifuge at 190×g for 3 minutes.
d	Aspirate and discard the supernatant, add 3mL of EuLV Medium I (with 6mM glutamine), and gently pipette to disperse the cells evenly.
e	Use a cell counter to determine the cell density and viability of live cells. Transfer all cells to a 125mL shake flask containing 27mL of medium. Label the resuspended cells as passage 1. **Acceptable standard: Cell viability ≥ 80%.**
f	Place the shake flask in a 37℃, 170 rpm, 8% CO_2 incubator for three days.

2.2.2. Passage

a	After three days of culturing, use a cell counter to determine the cell density and viability of live cells. **Acceptable standard: Cell viability ≥ 90%.**
b	Based on a concentration of 4×10^5 cells/mL, calculate the required volume of cell suspension for a 125mL shake flask (with a culture volume of 30mL) and transfer the cells to a centrifuge tube.
c	Centrifuge at 190×g for 3 minutes.
d	Aspirate and discard the supernatant, add 3mL of EuLV Medium I (with 6mM glutamine), and gently pipette to disperse the cells evenly.
e	Transfer all cells to a 125mL shake flask containing 27mL of medium.
f	Place the 125mL shake flask in a 37℃, 170 rpm, 8% CO_2 incubator for culture.

g	Repeat step 2.2.2 a-2.2.2 f to maintain or expand the cell culture scale.

2.2.3. Cryopreservation

a	Prepare a cryopreservation solution containing 10% DMSO by mixing medium and DMSO in a 9:1 volume ratio, and pre-cool it in a 2-8℃ refrigerator. **Note: The cryopreservation solution must be prepared fresh and used within 1 hour of preparation.**
b	After three days of cell culture, use a cell counter to determine the cell density and viability of live cells. **Acceptable standard: Cell viability ≥ 90%.**
c	Based on the requirement of 1.5×10^7 live cells per cryovial, calculate the total number of live cells needed and transfer the corresponding cell suspension to a centrifuge tube.
d	Centrifuge at 190×g for 3 minutes.
e	Aspirate and discard the supernatant. Resuspend the cells in an appropriate volume of cryopreservation solution, aliquoting 1mL per cryovial. **For example: To cryopreserve 5 cryovials, transfer a total of 7.5×10^7 live cells into a centrifuge tube, aspirate and discard the supernatant, and resuspend the cells in 5mL of cryopreservation solution.**
f	The steps are the same as described in 2.1.3 e-2.1.3 h.

2.3. Operating Suggestions

- Cells that have just been resuscitated should not be used for cell transfection. It is recommended to use cells between passage 3 and passage 20.
- During all cell operations, gently pipette to mix the cells. Avoid vigorous mixing or pipetting. The condition of the cells is critical for optimal experimental results.
- All preparation and incubation of the transfection reagent should be performed at room temperature.

2.4. Equipment Guide

- Ensure that the temperature of the equipment is properly calibrated. In some cases, the total heat generated by the shaking incubator may lead to slower cell growth, aggregation, or cell death.

- Ensure that the CO_2 concentration of the equipment is properly calibrated. The CO_2 concentration should be within the range of 8% ± 1%.

03 Lentiviral Vector Production

Seeding

Provide optimal density for adherent cells (target confluence of 60%-80%) to ensure sufficient surface area for transfection and lentiviral vector production. For suspension cells, provide optimal density and cell viability to enable efficient transfection and scalable lentiviral vector production.

Transient Transfection

Introduce a single transfer vector into the cells, simplifying the lentiviral vector production process and avoiding multi-plasmid co-transfection, while maintaining high titers and minimizing batch-to-batch variations.

Induction

Induction includes: (a) using an inducible promoter (such as tetracycline-responsive substances) to activate the lentiviral genes, or (b) applying enhancers to increase lentiviral titer by improving transfection efficiency and reducing cytotoxicity.

Harvesting

Collect the supernatant 48 hours after transfection to harvest the highest lentiviral titer, then centrifuge to remove cell debris for titer determination.

3.1. Adherent Cells

3.1.1. Seeding (10cm culture dish)

a	The steps are the same as described in 2.1.2 a-2.1.2 e.
b	Measure cell density and viability. Based on a live cell density of 4×10^5-5×10^5/mL and a culture volume of 10mL, calculate the required volume of cell suspension for a 10cm culture dish. **Acceptable standard: Cell viability ≥ 90%.**
c	Incubate the cells in a 37℃, 5% CO_2 incubator.

3.1.2. Transient Transfection

a	24 hours after seeding, observe cell confluence under a microscope, with the optimal range being 60-80%.
b	Prepare the transfection complexes according to the table below, and then scale the preparation in the correct proportion based on the number of 10cm culture dishes required.

Table 1: Transfection Complex Preparation Steps for 10 cm Culture Dish

DMEM Complete Medium	Plasmid	Transfection Reagent
Add up to 900μL	25μL	75μL

c	Mix the plasmid and reagents in the order shown in the table, incubate at room temperature for 15 minutes, then slowly add the transfection complex to the 10cm culture dish and gently shake the dish to ensure even distribution.
d	Incubate the cells in a 37℃, 5% CO_2 incubator for 6 hours.

3.1.3. Induction

a	Six hours after transfection, aspirate and discard the supernatant. Add 10mL of DMEM complete medium containing 10% EuLV Inducer, adherent.
b	Incubate the cells in a 37℃, 5% CO_2 incubator for 42 hours.

3.1.4. Harvest

a	Please refer to Section 3.3.

3.2. Suspension Cells

3.2.1. Seeding (125mL Shake Flask)

a	Repeat steps 2.2.2 a-2.2.2 f to complete cell seeding.

3.2.2. Transient Transfection

a	Three days after seeding, measure the live cell density and viability. **Acceptable standard: Cell viability ≥ 90%, live cell density: 4×10^6-6×10^6/mL.**
b	Gently invert the centrifuge tube containing EuLV Medium I and plasmid (as shown in the table below) 4-5 times to mix thoroughly.

Table 2: Transfection Complex Preparation for 125mL Shake Flask (30mL)

EuLV Medium I	Plasmid	Transfection Reagent
2.76mL	60μL	180μL

c	After adding Transfection Reagent to the mixture, vortex the centrifuge tube 4-5 times. **Note: If other culture systems are used, adjust the reagent amounts accordingly.**
d	Let the transfection mixture stand at room temperature for 15 minutes, then slowly add it to the shake flask.
e	Place the cells in a 37℃, 170 rpm, 8% CO_2 shaking incubator to continue culturing.

3.2.3. Induction

a	Six hours after transfection, add 8% EuLV PCL Inducer, suspension (e.g., for a 30mL production volume, add 2.4mL).
b	Place the cells in a 37℃, 170 rpm, 8% CO_2 shaking incubator to incubate.

3.2.4. Positive Rate Detection

a	Twenty-four hours after transfection, remove 200μL of cell suspension from the shake flask to detect the positive rate. **Acceptable standard: Positive rate ≥ 30%.**
b	Continue culturing the remaining cells for 24 hours.

3.2.5. Harvest

a	Please refer to 3.3.

3.3. Harvest Lentiviral Vector

a	Harvest the lentiviral vector 48 hours after transfection, collect the culture supernatant, and then centrifuge at 1,500×g for 10 minutes.
b	Immediately aliquot the lentiviral vector into 200μL portions in EP tubes and store at -80°C, or proceed with "Lentiviral Vector Titer Testing."

04 Lentiviral Vector Titer Testing

Important Note

Lentiviral vector handling must be done according to institutional guidelines. All materials must be treated with a 10% bleach solution after handling.

4.1. Operating Suggestions

- Gently pipette to mix the lentiviral vector. Do not vortex, and avoid vigorous mixing.
- Cells that have just been resuscitated should not be used for cell transduction. It is recommended to use cells between passage 3 and passage 20.

4.2. Transduce Jurkat Cells (Day 1)

a	On the day of transduction, measure the live cell density and viability of Jurkat cells. **Acceptable standard: Cell viability ≥ 90%.**
b	Add RPMI1640 complete medium (with 10% fetal bovine serum) to the centrifuge tube, dilute the Jurkat cells to a final live cell density of 2×10^5 cells/mL. Add 0.1% Polybrene (e.g., for a 15mL culture volume, add 15μL), and gently pipette to mix.
c	One hour before transduction, seed the Jurkat cells in a 24-well culture plate, with a culture volume of 500μL per well.
d	If using frozen lentiviral vector, please remove them in advance and allow them to thaw to room temperature. **Note: Do not expedite the thawing of the lentiviral vector, as this may reduce its titer.**
e	In each EP tube, sequentially dilute 150μL of lentiviral vector into 150μL of medium, preparing 12 consecutive dilution factors (2^1 to 2^{12}) (for lentiviral vector titer control).
f	In each EP tube, sequentially dilute 150μL of lentiviral vector into 150μL of diluted medium, preparing 9 consecutive dilution factors (2^1 to 2^9) (for lentiviral vector samples). **Note: If your lentiviral vector has been concentrated, additional dilution factors may be required.**
g	Transfer 100μL of the diluted lentiviral vector to the corresponding well of a 24-well plate to transduce the Jurkat cells.
h	Place the 24-well plate in a 37℃, 5% CO_2 incubator and incubate for 48 hours.

4.3. Titer Detection (Day 3)

a	Gently pipette to ensure the cells are evenly dispersed.

b	Process the cell samples using a flow cytometer.
c	Calculate the lentiviral titer.
d	Select a positive range of 5%-35%, and then determine the corresponding dilution factor based on the percentage of EGFP+ cells.

Table 3: Example of Lentiviral Titer Calculation

Lentiviral Vector Dilution Factor	EGFP$^+$ Positive Cell Rate
2^5	80%
2^6	60%
2^7	36%
2^8	18%

Based on Table 3 and the positive reference range, select the 2^8 dilution factor for calculation.

Use the following formula to calculate the titer:

Titer = (F×C/V)×D	F	EGFP$^+$ positive cell rate
	C	Number of cells per well at transduction
	V	Lentiviral vector volume(mL)
	D	Lentiviral vector dilution factor

Example:	F	18%
	C	100,000 (number of cells per well at infection)
	V	0.1 mL (100μL of medium)
	D	2^8
Calculation:		Titer = (0.18×100,000 / 0.1) × 2^8 = 4.6×10^7 TU/mL

05 Troubleshooting

Description		Recommendations
Low lentiviral titer	a	Cell quality assurance **Key: Focus on cell density and viability to ensure optimal cell conditions.**
	b	Reagent shelf-life management **Key: Validate reagent expiration (e.g., transfection efficiency >30%) and avoid using expired reagents to prevent batch failure.**
	c	Plasmid quantity optimization **Key: Adjust plasmid ratios (e.g., gene of interest: transfection reagent = 1:3, 1:2, 1:1) to achieve higher transfection efficiency.**
Cell quality assurance	a	Microbial contamination screening **Key: Perform systematic screening for bacteria, fungi, and mycoplasma contamination.**
	b	Differentiating between inducers and additives **Key: Pay attention to the order of use for inducers and additives.**
	c	Cell passage quality assessment **Key: Verify whether the passage number exceeds the prescribed limit, and if the cell density during passage culture exceeds 7×10^6 live cells/mL.**

06 Safety

6.1. Biosafety Requirements

Lentiviral vectors are classified as Biosafety Level 2 (BSL-2/P2) preparations. All operations, including waste disposal, must be conducted in a Class II biosafety cabinet (BSC) within a BSL-2 facility. Waste containers must be labeled with a biohazard symbol and sterilized by autoclaving (121°C, 30 minutes).

6.2. Toxicity Risk Warning

The toxicity of lentiviral vectors is inherently linked to the transgene carried in the transfer vector. For vectors encoding oncogenes (such as HER2, RAS mutants) or tumor suppressor genes (such as p53, PTEN):

- Waste must undergo chemical inactivation (e.g., 1% sodium hypochlorite for ≥1 hour) before autoclaving.
- Operators must wear double gloves and puncture-resistant lab coats, and monitor for symptoms within 72 hours after handling.

6.3. Measures for Accidental Leakage

a	Exercise caution to prevent splashing when handling lentiviral vectors. If the biosafety cabinet is contaminated with lentiviral vectors during the procedure, immediately wipe it clean with an inactivation solution containing 75% ethanol and 1% SDS. After coming into contact with lentiviral vectors, soak all pipette tips, centrifuge tubes, culture plates, and media in an inactivation solution containing 75% ethanol and 1% SDS.
b	Lentiviral vector-related waste must be specially collected and sterilized by autoclaving at high temperatures for uniform disposal.

EurekaBio

Shenzhen Eureka Biotechnology Co., Limited
Tel: +86-755-86562586
E-mail: enquiry@eurekabio.com
Add: 8F, City Garden Cyberport Building, No.1079 Nanhai Street, Nanshan District, Shenzhen, China