BlueKit™
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Ver202409

Instructions for Use of Lentiviral Packaging Kit

Cat.No. HG-HIV-CUL-001

The Reagent is intended for research use only, not for diagnostic purposes.

CONTENTS

1. Product Description

Lentivirus packaging is a widely employed technique to generate lentiviral vectors containing specific genes for applications such as gene function studies and cell or gene therapy. This process involves multiple steps, demanding precise execution and advanced experimental skills. Traditionally, adherent 293T cells are used as packaging cells, with lentivirus production initiated following co-transfection with a four-plasmid system. However, the adherent packaging system yields a limited amount of virus, which poses challenges for large-scale virus production .To address the issues of low virus yield and complicated packaging methods, Hillgene has developed a universal lentivirus packaging kit, drawing on years of expertise in lentiviral production. This kit utilizes a four-plasmid system (including the packaging plasmids gag/pol and rev, the envelope plasmid VSV-G, and the shuttle plasmid), and is designed with enhanced safety. The key innovation of this product lies in using suspended 293T cells for lentivirus packaging. Hillgene’ s proprietary serum-free suspension culture technology, combined with the HiLenti™ transfection reagent, enables the production of highly functional lentivirus in serum-free conditions, making it more suitable for scalable virus production with improved yields and reliability.

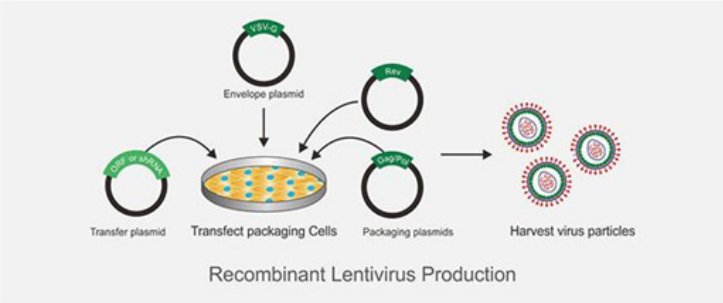

Figure 1. Lentivirus Vectors（LVVs） Packaging Principle

2. Production Composition and Introduction

Category	Production Name	Part Number	Specification	Quantity	Storage Conditon
Suspension system reagents	HiLenti™ 293T Suspension Cells Line	HG-HL-293T	1 Vial	1 piece	-80°C
	HiLenti™ 293T Suspension Medium	HG-MD001-1000	1 L/Bottle	2 bottles	2-8°C
	HiLenti™ Transfection Enhancer	HG-MD005-125	125 mL/bottle	1 Bottle	2-8°C
Universal Lentiviral Packaging Reagents	HiLenti™ Transfection Reagent	HG-HL-TD001	1 mL/Vial	2 Vial	-20°C
	HiPlas™ -LVV-T-OptiMix	HG-pHi005-R	1mg：1mL/ Vial	1 Vial	-80°C
	HiPlas™ -lenti-GFP	HG-pHi006	1mg：1mL/ Vial	1 Vial	-80°C
	HiLenti™ Lentiviral Storage Medium	HG-LPC001	125 mL/ Bottle	1 Bottle	2-8°C

2.1. HiLenti™ 293T suspension cells Line:

HiLenti™ 293T suspension cells are derived from ATCC's 293T cell line and are adapted for suspension culture in HiLenti™ 293T suspension medium after acclimation. Cell line characteristics: (1) expresses E1A adenovirus gene; (2) expresses SV40 large T antigen; (3) approximately 24 hours cell doubling time; (4) has a high lentiviral production

lidacity between cell passages 5-15.

2.2. HiLenti™ 293T Suspension Medium

HiLenti™ 293T Suspension Medium is a chemically defined medium developed for HiLenti™ 293T suspension cells that is free of animal origin, serum, and protein.

2.3. HiLenti™ 293T Transfection Enhancer

HiLenti™ 293T Transfection Enhancer can help the assembly and secretion of lentivirus after lentiviral packaging in HiLenti™ 293T suspension cells, thereby increasing the yield of lentivirus

2.4. HiLenti™ Transfection Reagent

HiLenti™ Transfection Reagent is a pure chemically synthesized, animal-derived component-free transduction reagent suitable for co-transfection of multiple plasmids and production of viral vectors through this method.

2.5. HiPlas™-LVV-T-OptiMix

HiPlas™-LVV-T-OptiMix is a plasmid developed by Hillgene by mixing three plasmids, HiPlas™-lenti-VSVG, HiPlas™-lenti-Gag/pol, and HiPlas™-lenti-Rev, in a certain proportion and can be used directly.

2.6. HiPlas™-lenti-GFP

HiPlas™ -lenti-GFP is a plasmid that expresses GFP fluorescent protein on the Hillgene vector backbone. The gene to be expressed can be cloned into the HiPlas™ -lenti vector of HiPlas™ -lenti and packaged with the corresponding virus. It can also be used as a positive control for virus packaging.

3. Required but not Provided

Lentivirus packaging is a complex, time-consuming process that requires a range of equipment and materials. Below is a list of the essential items for virus packaging experiments.

3.1. Instrument List

Instrument List
Shaking incubator
Fluorescence cell analyzer
Biosafety cabinets
Electric constant temperature water bath
Desktop refrigerated centrifuge

3.2. Material List

Material List
Glutamine
Dimethyl sulfoxide
125mL triangular shake flask
250mL triangular shake flask
500mL triangular shake flask

1L triangular shake flask
1.5L triangular shake flask
3L triangular shake flask
5Lcell shake flask
5mL pipette
10 mL pipette
25mL pipette
50mL pipette
200uL Filter tip
1000uL Filter tip
250mL large capacity conical flask centrifuge tube
500mL large capacity conical flask centrifuge tube
2000mL large capacity conical flask centrifuge tube
50mL centrifuge tube
1.5mL cryovial

4. General Considerations and Biosafety Guidelines

4.1. For your and others safety, it is important to fully understand the potential hazards of using lentiviruses and the necessary precautions to produce and use them in the laboratory. The National Institutes of Health and the Centers for Disease Control have classified lentiviruses as Biosafety Level 2 hazardous biological products.

4.2. Please follow Biosafety Level 2 (BSL-2) for the production and use of lentivirus. The following are some key protective measures commonly taken by BSL-2 laboratories:

- Training: All laboratory personnel must receive biosafety training to understand the characteristics of the pathogens they handle and the associated risks.
- Personal Protective Equipment (PPE):
- Laboratory workers should wear appropriate personal protective equipment, such as lab coats, gloves, masks, goggles or face shields.
- Biological safety cabinet: Use a biosafety cabinet for operations involving infectious materials to prevent the generation and spread of aerosols.
- Laboratory design: The laboratory should have clear entrances and exits, restrict access to unauthorized personnel, and be equipped with hand washing facilities.
- Waste disposal: All infectious waste should be placed in dedicated, biohazard labeled containers and disinfected and disposed of in accordance with regulations.
- Equipment and surface Disinfection: Regularly disinfect laboratory equipment and surfaces to reduce the risk of cross-contamination.
- Sharps disposal: Use dedicated sharps boxes to collect needles, blades and other sharp objects to avoid accidental injuries.

- Operating procedures: Follow strict operating procedures, such as avoiding contact with the mouth when using a straw, and avoid eating, drinking or using personal items in the work area.
- Emergency plans: Develop and be familiar with emergency plans to respond to possible unexpected incidents such as chemical spills, fires, or personal exposures.
- Health monitoring: Laboratory workers should undergo regular health checks, especially for pathogens workers may be exposed to.
- Waste decontamination: All potentially contaminated waste should be appropriately decontaminated before leaving the laboratory.
- Records and Reports: Maintain detailed laboratory records including all pathogens used, experimental procedures, and any accidents or exposures.
- Safety signs: Set up obvious biohazard signs at the entrance and exit of the laboratory to remind personnel to pay attention to safety.

5. Lentiviral Preparation

5.1 NK Cell Preparation Flowchart

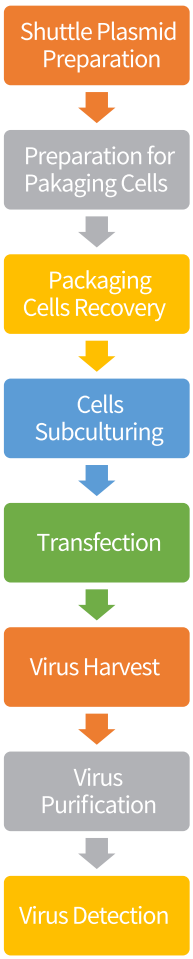

Figure 2. LVVs Packaging Flowchart

5.2. Shuttle Plasmid Preparation

5.2.1. The HiPlas™-lenti-GFP plasmid product is provided in the lentiviral packaging kit. This product is designed based

on public information and plasmid backbones have demonstrated excellent performance after long-term validation by Hillgene. Users can clone the sequence to be expressed into the vector. The multiple cloning restriction sites (BamHI+SalI) are as follows:

5.2.2. Users can design and construct shuttle plasmids by themselves. The lentiviral packaging kit provides HiPlas™-LVV-T-OptiMix plasmid products, which can be used together with the shuttle plasmid for plasmid transfection operations.

5.3. Preparation for Packaging Cells

The HiLenti™ 293T suspension cell product is included in the lentiviral packaging kit, and it performs well in LVV packaging. Users can establish a cell bank in the laboratory following the method provided in the instructions, using the HiLenti™ 293T suspension cell product from the kit.

5.3.1. Cell bank construction process

5.3.2. Definition of cell lines and cell banks

5.3.2.1. The received HiLenti™ 293T suspension cells were defined as the P0 generation of 293T, and the cells were subcultured according to a fixed subculture ratio, with each subculture considered as 1 generation.

5.3.2.2. The cell bank management follows the three-level management, namely the primary cell bank (PCB), the master cell bank (MCB) and the working cell bank (WCB). The PCB is used to establish the MCB, and the MCB is used to establish the WCB.

5.3.3. Solution preparation

Process Steps	Solution Name	Reagent Name	Concentration
Cell recovery and expansion	Recovery culture medium	HiLenti™ 293Tsuspension medium	/
		200 mM Glutamine	6 mM/L
Cell cryoperservation	2×Cell cryopreservation solution	Recovery culture medium	840 mL/L
		Dimethyl sulfoxide (DMSO)	160 mL/L

5.3.3.1. Preparation method of 1L recovery culture medium

In a biosafety cabinet, aseptically pipette 30 mL of glutamine solution (200 mmol/L) into 970 mL of HiLenti™ 293T suspension medium, mix well, and use immediately.

5.3.3.2. Preparation method of 1L Cell cryopreservation solution

In a biosafety cabinet, aseptically take 160 mL of dimethyl sulfoxide and add it to 840 mL of sterile recovery culture medium. Mix thoroughly and use immediately.

5.3.4. HiLenti™ 293T suspension cell recovery

5.3.4.1. Set the water bath temperature to 37.0 °C and keep it constant for later use;

5.3.4.2. Preheat the seed culture medium in a 37.0 °C constant temperature water bath for ⩾ 20 min;

5.3.4.3. Set the shaker parameters (amplitude 50 mm): culture temperature 37.0 °C , CO_2 Concentration 6.0%, 130rpm;

5.3.4.4. In a biosafety cabinet, pipette 9 mL of pre-warmed recovery culture medium into a 15 mL centrifuge tube;

5.3.4.5. Immediately place the a cryovial containing cells in a 37.0 °C water bath to thaw quickly. Do not allow the water surface of the water bath to submerge the tube openings of the cryovial. Thaw quickly within 2 minutes;

5.3.4.6. In the biosafety cabinet, open the cryovial lid and use a 5 mL sterile pipette to transfer all the cell suspension in the cryovial to a 15 mL centrifuge tube filled with preheated recovery culture medium. After tightening the lid, take the tube out of the biosafety cabinet and centrifuge at 160 g for 5 minutes;

5.3.4.7. In a biosafety cabinet, open the centrifuge tube lid, discard the supernatant in the centrifuge tube into a 50 mL centrifuge tube, and resuspend the cell pellet in 5 mL preheated seed culture medium to form a suspension;

5.3.4.8. Transfer the cell suspension to a 125 mL disposable sterile conical flask and add 25 mL of preheated seed culture medium. After mixing, pipette 0.5 mL of cell suspension for counting. The cell viability should be ⩾ 80%;

5.3.4.9. After labeling the outer wall of the culture shake flask, place it in a shaking incubator at 37.0 °C , 6.0% CO_2, and 160 rpm. Record the start time and incubate for 3 days;

5.3.5. Cell expansion Ⅰ , Ⅱ , Ⅲ , Ⅳ generation;

5.3.5.1. Set the water bath temperature to 37.0 °C and keep it constant for later use;

5.3.5.2. Preheat the seed culture medium in a 37.0 °C constant temperature water bath for ⩾ 20 min;

5.3.5.3. Set the shaker parameters (amplitude 50 mm): culture temperature 37.0 °C , CO_2Concentration 6.0%, 160 rpm;

5.3.5.4. Disinfect the seed culture medium and the outer surface of the culture shake flask with 75% alcohol and place them in a biosafety cabinet. Wipe the seed culture medium and the outer surface of the culture shake flask with 75% alcohol in the biosafety cabinet;

5.3.5.5. Gently shake the flask to mix the cells, then aseptically pipette 0.5 mL of cell solution, count the cells, and test the cell density and viability. Seed cell shake flask expansion I, II, III, and IV generations must meet the requirements of cell viability ⩾ 90% and cell density of (1.5-7.0)$\times 10^6$ cells/mL;

5.3.5.6. The planned subculture density is 0.5$\times 10^6$ cells/mL, the operating range is (0.4~0.6) $\times 10^6$ cells/mL;

5.3.5.7. According to the cell test results and the planned subculture volume (cell expansion I generation: 60±10 mL; cell expansion II generation: 120±10 mL; cell expansion III generation: 300±50 mL; cell expansion IV generation: 600±50 mL), calculate the required volume of cell suspension and the volume of fresh seed culture medium to be added according to the formula;

5.3.6. Volume calculation:

5.3.6.1. Required cell suspension volume (mL) = planned subculture volume (mL) × planned seeding density / viable cell density

5.3.6.2. Volume of seed medium added (mL) = planned subculture volume (mL) - required cell suspension volume (mL)

5.3.6.3. According to the calculation results, in the biosafety cabinet, aseptically transfer the required cell solution and the fresh seed culture medium to be added into a disposable sterile shaking flask and mix well

5.3.6.4. Label the cell suspension name, generation number, culture volume, batch number, and operation date on the outer wall of the culture shake flask. Culture cell in a shake incubator with 6.0% CO_2 and 160 rpm, record the start time of culture, and culture time is 3 ± 1 days;

5.3.7. Cell cryopreservation

5.3.7.1. Confirm that the gradient cooling box is ready;

5.3.7.2. Prepare sterile pipettes, 500 mL disposable sterile conical flasks, 2000 mL disposable sterile conical flasks, 50 mL centrifuge tubes, cell cryopreservation tubes, cryopreservation boxes, and sterile gloves, disinfect them with 75% alcohol, and place them in a biosafety cabinet;

5.3.7.3. Turn on the UV sterilizer of biosafety cabinet. After sterilization for ⩾ 30 minutes, turn on the fan and run it for ⩾ 30 minutes.

5.3.7.4. Prepare cryovial labels;

5.3.7.5. Prepare 200 mL of cell cryopreservation solution × 2;

5.3.7.6. Confirm the planned number of cryovials, calculate the number of cryovials (planned number of cryovials + 10) and the cryopreservation volume;

5.3.7.7. According to the formula: Cryopreservation solution volume × 2 = cryopreservation volume × calculated number of cryovials ÷ 2, calculate the required cryopreservation solution volume, and pipette the corresponding amount of cell cryopreservation solution into a 500 mL disposable sterile conical flask, and place it in a 4 °C environment for precooling.

5.3.7.8. Disinfect the outer surface of the disposable sterile conical flask containing IV generation cell expansion with 75% alcohol and transfer it to the biosafety cabinet. In the biosafety cabinet, gently shake the cell suspension to mix evenly, then aseptically pipette1 mL of the suspension for cell counting. The cell density should be in the range of (3.0~7.0)×10^6 cells/mL, with viability ⩾ 90%, meeting the required standards;

5.3.7.9. According to the formula, the total number of living cells in the shake flask is calculated = the density of living cells in the shake flask × the volume of cell fluid in the shake flask;

5.3.7.10. Note: The volume of cell solution in the shake flask = the volume of cell solution at the time of inoculation - 10 mL (10 mL is the loss of cell solution volume)

5.3.7.11. According to the formula, the number of living cells required = cryopreserved living cell density × cryopreserved volume × calculated number of cryopreserved tubes × 120%, calculate the number of living cells required for cryopreservation. Cryopreserved living cell density: 1.0×10^7cells/mL; Cryopreservation volume: 1.0 mL/vial, 20% more cells were taken out during cryopreservation as cryopreservation operation loss;

5.3.7.12. According to the formula: Cell solution volume required for cryopreservation = number of living cells required for freezing / cell solution density, calculate the cell solution volume required for cryopreservation

5.3.7.13. In a biosafety cabinet, use a sterile pipette to transfer the required cell solution in 250 mL or 500 mL sterile centrifuge tubes. The volume of each centrifuge tube should be ⩽ 80% of the total volume of liquid, and the tube should be taken out of the biosafety cabinet after tightening the lid. After the centrifuge tube is taken out of the biosafety cabinet, centrifuge at 160 g for 5 minutes;

5.3.7.14. Discard the supernatant in a sterile shake flask in a biosafety cabinet, resuspend the centrifuged cells in cell culture medium, and transfer to a 500 mL disposable sterile conical flask to prepare a batch of homogenized cell suspension. Pipette 1 mL of sample for cell counting, which should meet the following requirements: cell density range is (1.6-2.4) ×10^7cells/mL, cell viability ⩾ 90%;

5.3.7.15. Slowly add an equal volume of cell cryopreservation solution× 2 to the cell suspension, record the volume of the cell suspension, the volume of cell cryopreservation solution used and the time of addition, gently mix the cell suspension, and pipette 1 mL of sample for cell counting, which should meet the following requirements: cell density range is (0.8-1.2) ×10^7cells/mL, cell viability ⩾ 90%;

5.3.7.16. Open the cryovial lip and transfer the cell suspension to the cryovial. The volume of cell suspension in each cryovial is 1.0 mL. Cover the cryovial lid and insert it into the cryopreservation box in turn;

5.3.7.17. Transfer the cell cryopreservation box to an ultra-low temperature refrigerator (temperature ⩽ -80 °C) and record

the time of entering the refrigerator

5.3.7.18. Record the time from cell resuspension to storage in the refrigerator. The time from resuspension to storage in the refrigerator must be ⩽ 90 minutes.

5.3.7.19. The cells were stored in an ultra-low temperature refrigerator (temperature ⩽ -80 °C) for 24±2h, and the cooling boxes were taken out from the ultra-low temperature refrigerator in order from the front to the back according to the number, and the removal time was recorded. In the dry ice box, the cell line cryopreservation tubes in the cell cryopreservation box were transferred to the liquid nitrogen cryopreservation box in sequence according to the serial number;

5.3.8. Initial testing for cell bank

After the original cell bank is established, samples are taken for sterility, mycoplasma testing and preliminary subculture stability studies. If the test results are negative, it can be used.

5.3.9. Initial subculture stability study

After the cell bank was placed in liquid nitrogen for 24 hours, the frozen cells were taken for primary cell bank subcultre stability study. After cell recovery, the cells were subcultured 3 to 5 times continuously, with an inoculation density of 0.5×10^6 cells/mL, and subcultured every 2 to 4 days. The shake flask specification was a 125mL disposable sterile conical flask with a culture volume of 30 mL During the cell subculture period (excluding recovery), the cell doubling time met 12 to 20 h, the cell density met $(1.5 \text{ to } 7.0)\times10^6$ cells/mL, and the cell viability was ⩾ 90%, which was considered to be stable cell growth.

6. Lentivirus Packaging

6.1. Preparation before packaging cell recovery:

Place the culture medium in a 37 °C water bath to preheat (or place it at room temperature for more than 30 minutes in advance); turn on the biosafety cabinet for UV disinfection for 30 minutes, and then blow it for self-cleaning for 15 minutes; Take a 50 mL centrifuge tube and add 9 mL HiLenti™ 293T suspension culture medium for use.

6.2. Cell thawing and recovery

Quickly collect cryopreservation cells from the cell bank using a pre-cooled cryopreservation box or dry ice, and quickly transfer them to the cell culture room. Check whether the cryopreservation tube label is intact and whether the cryopreservation tube is broken. Use hemostats to clamp the cryopreservation tube cover, and slowly insert the cryopreservation tube into a water bath that has been preheated to 37 °C for thawing. Make sure that the cryopreservation tube cover is not immersed below the water level of the water bath. Use hemostats to clamp the cryopreservation tube, and slowly draw wavy lines in the water bath to thaw (the thawing time should not exceed 120 seconds). When the cells in the cryopreservation tube melt until only a small ice ball remains, remove it from the water bath.

6.3. Centrifuge and resuspend

Disinfect the surface of the cryovial with 75% ethanol and quickly transfer the cells into a 50 mL centrifuge tube containing 9 mL of HiLenti™ 293T suspension medium. Centrifuge at 160 g for 5 minutes at room temperature, carefully discard the supernatant, and resuspend the cells in 20 mL of HiLenti™ 293T suspension medium. After adding the medium, gently tap the bottom of the centrifuge tube with your fingertips to loosen the cell pellet, then slowly pipette the suspension to ensure uniformity.

6.4. Fluid replenishment count:

6.4.1. Transfer the cell suspension to the prepared 125 mL sterile shake flask, add 200 mM glutamine solution at a final concentration of 6 mM, shake well, and take samples for counting.

6.4.2. The flask was placed in a carbon dioxide shake incubator for shaking culture at 37 °C and 6% CO_2, 130 rpm, and the culture time is 2~3 days.

6.5. Subculturing:

6.5.1. First generation subculture:

6.5.1.1. After 2-3 days of recovery culture, take the flask to be subcultured out of the CO_2 incubator and put it in a biosafety cabinet. Shake the flask to make the suspension uniform and pipette about 0.5 mL of cell suspension was used for cell counting, and the testing cell density, cell viability, and clumping rate were determined using a cell counter.

6.5.1.2. After counting is completed, press 0.5×10^6cells/mL Calculate the total volume of cell culture medium, shake the seed solution shaker to make the seed solution uniform, use a sterile pipette to draw the required volume of cell suspension and add it to the shaker, shake the shaker to make the cell suspension uniform, add 200 mM glutamine at a final concentration of 6 mM (ignore the volume of 200 mM glutamine solution added in the calculation). Place the subcultured cells in a carbon dioxide incubator for 2-3 days. Culture conditions: 37 °C , 6% CO_2, 130 rpm.

6.5.2. Continue subculture

According to the packaging volume of different schemes, perform multiple subcultures according to the previous step to obtain a suitable volume of cells (currently the packaging volume of the shake flask is fixed at 300mL or 2L), and the cell density should be $(5.0\text{-}7.0)\times10^6$ cells/mL, and the cell viability should be ⩾ 90%, then transfection can be performed. (Refer to the table below for subculture volume and shake flask specifications)

Subculture Volume	Culture Container
20mL	125mL Flask
50mL	250mL Flask
300mL	1500mL Flask
500mL	1500mL Flask
2000mL	5L Flask

6.6. Lentivirus packaging protocol

6.6.1. Preparation of cell suspension:

Disinfect the outer surface of the shake flask containing the cell suspension with 75% alcohol, then transfer it to the biosafety cabinet. Shake the flask gently to ensure uniform suspension of the cells. Pipette 0.5 mL of the cell suspension for counting and use a cell counter to measure live cell density, viability, and aggregation rate. Confirm that the cell density is between $(5.0\text{-}7.0)\times10^6$ cells/mL and that the cell viability is ⩾ 90%. Once these criteria are met, proceed with lentiviral packaging.

6.6.2. Transfection Complex Preparation:

6.6.2.1. Pipette 10% of the total culture volume of HiLenti™ 293T suspension medium and divide it equally into two centrifuge tubes or cups. Label one tube as "HiLenti™ 293T Suspension Medium" and the other as "DNA".

6.6.2.2. Plasmid Addition: Using the CAR-T platform plasmid ratio of HiPlas™-LVV-T-OptiMix: vector plasmid = 13:7 (or other specified ratio), aseptically add the plasmids to the DNA tube. Shake gently and let the mixture stand at room temperature for 5 minutes.

6.6.3. Transfection Reagent Addition:
Calculate the required amount of transfection reagent based on the ratio of HiLenti™ transfection reagent: DNA = 2:1. Pipette the corresponding volume of HiLenti™ transfection reagent into the "HiLenti™ 293T Suspension Medium" tube and mix well.

6.6.4. Transfection Complex Mixing:
After the DNA solution has rested, pipette the contents of the "PEI" tube into the "DNA" tube while gently shaking the "DNA" tube. Once all the solution has been added, close the tube and shake to mix thoroughly. Let the mixture stand at room temperature for 15 minutes.

6.6.5. Transfection:
Once the transfection complex is ready, add it to the appropriate shake flask. Label the flask with the packaging batch number, shake to mix the contents, and return the flask to the incubator under the appropriate culture conditions (37 °C , 6% CO_2 , 130 rpm).

6.6.6. Post-Transfection Feeding:
6 to 8 hours after transfection, add 20% of the packaging volume (e.g., 400 mL for 2 L packaging) of HiLenti™ Transfection Enhancer and 2% of the packaging volume of 200 mM glutamine solution (e.g., 40 mL for 2 L packaging) to each flask in the biosafety cabinet.

6.6.7. Harvesting:
48 hours after transfection, take samples for cell counting and record cell density, viability, and aggregation rate. Centrifuge the suspension at 4000 rpm for 5 minutes, then collect the supernatant. Store the samples at -80 °C after testing.

7. Lentivirus Purification (Centrifugation and Resuspension Process)

7.1. Virus Collection:
Dispense the crude viral solution into centrifuge bottles and balance them using an electronic balance. Place the balanced bottles in a centrifuge set to 4000 g at 22 °C and centrifuge for 5 minutes. After centrifugation, discard the precipitate and retain the supernatant.

7.2. High-Speed Centrifugation:
Transfer the crude supernatant into 50 mL centrifuge tubes and balance using an electronic balance. Place the balanced tubes in a centrifuge set to 18,000 g at 22 °C for 1 hour. After centrifugation, slowly discard the supernatant and retain the precipitate.

7.3. esuspension:
Resuspend the virus pellet obtained from step 7.2 in 5-7 mL of HiLenti™ Lentiviral Storage Buffer. Mix thoroughly to create the virus concentrate.

7.4. Sterile Filtration:

7.4.1. Prepare all necessary materials in advance by placing them in the biosafety cabinet. Turn on the UV light for disinfection and self-purification for 30 minutes, then allow the cabinet to blow air and self-purify for an additional 20 minutes;

7.4.2. In the biosafety cabinet, sterilize the virus concentrate by filtering it through a 0.22 μm filter to obtain the virus stock solution.

7.5. Formulation aliquoting:

7.5.1. In a biosafety cabinet, add 20% human albumin at a volume ratio of 9:1 (virus stock solution/human albumin), and

the final human albumin ratio is 2%;

7.5.2. In a biosafety cabinet, aliquoting the LVVs solution into the cryovials.

7.6. LVV Testing:

After virus collection, perform necessary testing, including physical titer (p24), transduction titer, host protein residue, host DNA residue, endotoxin, and sterility. Once testing is complete, the lentivirus stock can be used for subsequent cell transduction. After LVV testing is complete, the LVVs can be used for cell transduction.

8. Troubleshooting

Problem Description	Possible causes	Solution
Low cell viability	The amplitude of the shaker is not appropriate	Adjust the speed or contact the manufacture for consultation
Low virus titer	GOI sequence is too long or complex in design	Contact the manufacture for consultation

9. Related Products

Product Name	Product Number
Host cell Residual DNA (Magnetic Bead Method) Sample Pretreatment Kit	HG-CL100
Lentiviral Titer p24 ELISA Test Kit	HG-P001L
293 HCP ELISA Kit	HG-HCP001
Human Residual DNA Detection Kit (qPCR- Fluorescent Probe Method)	HG-HD001
Human Residual RNA Detection Kit (RT-PCR- Fluorescent Probe Method)	HG-HR001
Human Residual Fragment Analysis Detection Kit (qPCR-- Fluorescent Probe Method)	HG-HF001
E1A&SV40LTA Residual DNA Detection Kit (Multiple qPCR- Fluorescence Probe Method)	HG-EA001
Nuclease ELISA Detection Kit	HG-BE001
Plasmid residual DNA detection kit (qPCR-fluorescent probe method)	HG-ZL001
BSA ELISA Test Kit	HG-BS001
Trypsin ELISA Kit	HG-TR001
BCA Rapid Protein Quantitation Assay Kit	HG-BC001
Lentiviral Packaging Kit	HG-HIV-CUL-001
HiPlas™ -LVV-T-OptiMix (ready-stock plasmid)	HG-pHi005-R
CD-19 CAR-T Spot Lentivirus	HG-CT1901
CD-19 CAR-NK Spot Lentivirus	HG-CN1901

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