Lentiviral Packaging Kit

Product Info

Ubigene Lentivirus Packaging Kit uses a third-generation packaging system in which the 3 'LTR of the genome is mutated to form self inactivation (SIN), whereby the viral genome, when integrated into the cell genome, does not produce new progeny virus and therefore has a relatively good safety profile. **The kit is composed of a lentiviral packaging plasmid mix (LV PacMix), control lentiviral plasmids carrying EGFP and Puromycin resistant gene, and Polybrene**, and can be used for efficient packaging and purification of high titer lentiviruses for use in vitro and in vivo experiments.

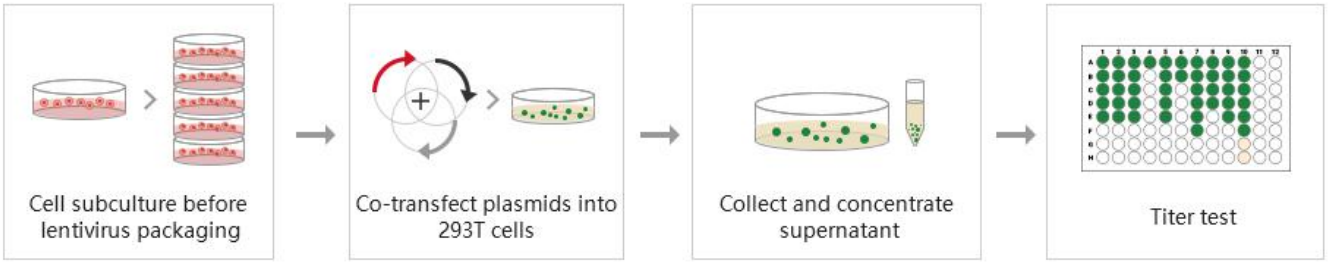
LV PacMix contains 3 helper plasmids for lentivirus packaging, which achieved efficient improvement of virus yield with better virus packaging effect by employing Ubigene exclusive developed formula. Both viral helper plasmids and control viral plasmids are the ready-to-use endotoxin-free plasmids, which can be used directly for transfection. If needing expression plasmids (lentiviral backbone), feel free to contact Ubigene for ordering.

In addition, Ubigene provides high-quality expression plasmids and 293T packaging cells, in which the 293T packaging cells of Ubigene has low passage, strong viability, can grow rapidly and have stronger survival ability, while being highly transfectable, and the yield of lentivirus is higher than that of a common 293T cell line. Feel free to contact Ubigene for ordering our 293T packaging cell line.

Kit Components

Catalog	Components	Size	Storage
YK-LVP-05	LV PacMix (endotoxin-free)	100 μL	-20℃
	LV GFP Ctrl (endotoxin-free)	100 μL	
	Polybrene (0.5 mg/mL)	1 mL	
YK-LVP-20	LV PacMix (endotoxin-free)	400 μL	-20℃
	LV GFP Ctrl (endotoxin-free)	100 μL	
	Polybrene (0.5 mg/mL)	1 mL	
YK-LVP-40	LV PacMix (endotoxin-free)	800 μL	-20℃
	LV GFP Ctrl (endotoxin-free)	100 μL	
	Polybrene (0.5 mg/mL)	2 mL	

Work Flow of lentiviral packaging



Protocol

1. Preparation

1.1 Cell Culture

Cell line: 293T cell line (Ubigene’ s 293T packaging cell line is recommended, Catalog#YC-A006)

Complete medium: 90%DMEM(high glucose)+10%FBS

Other reagents: 0.25% Trypsin, PBS buffer

Other materials: Culture plate or flask

1.2 Lentivirus packaging preparation

Reagents: Opti-MEM medium, Lipofectamine transfection reagent (e.g. lipofectamine 2000), or calcium phosphate transfection reagent

Reagents for lentivirus concentration: 50% PEG 6000, 2×HBSS

Other materials: 50ml centrifuge tube, 0.45μm syringe filter, syringe

1.3 Titer test preparation

Cell line: 293T cell line (Ubigene' s 293T packaging cell line is recommended, Catalog#YC-A006), or H1299 cell line

Reagents: Genome extraction kit, qPCR reagent, lentivirus standard reference

2. 293T Cell culture

2.1 Cell Thawing

① Take the cryopreserved cells from liquid nitrogen and transfer to a dry ice box.

② Warm up the complete medium in 37°C water bath

③ Pipette 6-7 ml of complete medium into a 15 ml centrifuge tube

④ Take out the cells from the dry ice box, shake gently to remove residual dry ice. Then hold the cap with forceps, quickly thaw cells in a 37°C water bath by gently swirling the vial to completely thaw the cells in about 1 minute (Note: keep the cells in the cryopreservation tube below water surface but the cap out of the water)

⑤ Inside the ultra-clean bench, sterilize the outer surface of the vial by wiping with an alcohol cotton pellet and leave it to dry. Transfer the thawed cells to the 15ml centrifuge tube containing complete medium by pipette, close the lid, and centrifuge at 1100 rpm for 4 mins at room temp to collect the cells.

⑥ Inside the ultra-clean bench, carefully remove and discard the supernatant. Resuspend cell pellet with 1ml of fresh complete medium and then transfer to a T25 flask containing 4 ml of complete medium, label the flask with cell name, date and passage no., incubate the flask in a 37℃, 5% CO^2 incubator.

2.2 Cell Passaging

① As long as the cells are 75%-85% confluent, it is ready to passage. Inside the ultra-clean bench, remove and discard the medium from the flask and briefly rinse the cell 1-2 times with 1×PBS (2-3 ml for T25 flask, 4-5 ml for T75) to remove residual medium and serum (serum contains trypsin inhibiting factor), but try not to wash down the 293T cells during washing.

② Add 1 ml of trypsin solution to flask and allow trypsin completely cover the cells (as shown Table1), until the majority of the cells become round and non-adherent as observed under the microscope, a large number of cells detached from each side when gently shaking and tapping the flask, terminate trypsin digestion immediately. If most of the cells remain adherent to the wall, appropriately extend trypsin incubation time or place the cells in the incubator for digestion.

③ Add complete medium to stop digestion, the volume is 2 times of trypsin. Then gently pipet the cells several times to allow all cells to be completely detached from the flask. (Note: Gently pipette the cells, try not to make bubbles or as less as possible)

④ Transfer the cell suspension with a 10 ml pipette into a 50 ml centrifuge tube, rinse the residual cells from the flask using appropriate volume of PBS , then collect and put them together to the 50 ml centrifuge tube. Close the cap and label.

⑤ Centrifuge at 1100 rpm for 4 mins at room temp. After centrifugation, remove and discard the supernatant and resuspend the cells with 2 ml of complete medium.

Table 1. Volume of Trypsin solution added to different size of culture plates/flasks

Size of culture plates/flasks	Trypsin Volume added
T25 flask	0.3 mL-0.5 mL
T75 flask	1 mL-1.5 mL
T175 flask	3-4 mL
10 cm plate	1 mL-1.5 mL
15 cm plate	3 mL-4 mL

⑥ Take 20 μL cell suspension for cell counting, inoculate the cells according to the cell density in Table 2

Table 2. Volume of complete medium to different size of culture plates/flasks

Size of culture plates/flasks	Cell amount for inoculation	Total volume of culture solution
T75culture flask	4.0×10^6/flask	12 mL
T175culture flask	9×10^6/flask	25 mL
10 cm plate	5.0×10^6/plate	10 mL
15 cm plate	1.2×10^7/plate	25 mL

⑦ Label the flask with cell name, date and passage no., incubate the flask in a 37℃, 5% CO^2 incubator.

3. Lentiviral Packaging

The following operations are based on using 10 cm culture plate for GFP lentivirus packaging (the dosage for using 15 cm plate should be correspondingly increased to 2.25 times that for using 10 cm):

① The day before virus packaging, inoculate 293T cells into a 10 cm plate, and the number of seeded cells should be optimal to achieve 80% ~ 90% confluence by cell growth on the day of packaging.

② 1 hour before packaging, take the cells from the incubator and change the medium to 8-10 mL Opti-MEM medium and place into the incubator to equilibrate.

③ Prepare the mixture of DNA-Lipofectamine 2000:

Solution A: Take 20 μL LV PacMix, mix with 20 μL LV GFP Ctrl (at a concentration of 500 ng/uL), and made up to 1.5 mL with Opti-MEM, mixing by inversion.

Note: For lentiviral expresion plasmids, it is recommended to add 10~12 μg.

Solution B: Take 60 μL Lipofectamine2000 and mix with 1.44 mL Opti-MEM, well mixing by inversion.

After incubation for 5 min at room temperature, mix solution A with solution B by inversion (avoid shaking) and stay for another 20-30 min at room temperature to form

DNA-Lipofectamine2000 mixture.

④ Add the DNA-Lipofectamine2000 mixture dropwise to 293T cells, gently shake the culture plate back and forth to well mix the mixture. And place the plate in a 37℃, 5% CO^2, saturated humidity incubator to incubate the cells.

⑤ 4-6 hours after transfection, change the medium to complete medium and incubate in a 37℃, 5% CO^2, saturated humidity incubator for 48 hours.

4. Viral supernatant collection

① After 48-hour incubation, collect the virus containing supernatant into a 50 mL centrifuge tube and centrifuge at 1500 rpm for 10 min.

② Filtrate the supernatant with a 0.45 μm syringe filter to remove the cell debris, and the lentivirus is in supernatant.

The lentivirus can be directly used for cell infection, if not used immediately, aliquot and store below -70℃.

5. Virus concentration

The crude lentivirus can be greatly improved the viral titer and purify after concentration and purification, which can infect the cells more efficiently.

① Add 50% PEG 6000 solution at 1/5 of the volume of the filtrate, mix thoroughly by inversion up and down, and place at 4℃ overnight.

② On the following day, centrifuge 1500 × g at 4℃ for 30 mins, discard the supernatant within the ultra-clean table.

③ Remove the supernatant completely with a pipette, and add 300-500 μL 2× HBSS to each 10 cm plate to resuspend the virus.

④ Aliquot the virus and store below -70℃.

6. Viral titer detection

Take the fluorometric counting method for example:

① The day before viral titer detection, inoculate the 293T cells into a 96-well plate at 5×

10^3 293T cells/ well (Volume of 100μL), 10 wells will be required for each type of virus.

② Prepare 6 sterile EP tubes, add 90 μL DMEM complete medium to each tube, take 10μL of the virus (to be assayed) into tube ①, and after mixing, take 10 μL from tube ① to tube ②, and continue the same operation until the last tube.

③ Aspirate the used medium from the plate and add 100 μL diluted virus containing medium solution to each well: the well corresponding to tube ① is marked as "10^{-1}" , the well corresponding to tube ② is marked as "10^{-2}" ..... the well corresponding to tube ⑥ is marked as "10^{-6}"

④ After 48 hours of incubation at 37℃, 5% CO^2, add 100 μL fresh DMEM complete medium to continue the incubation.

⑤ After another 24 hours, replace the medium with 150 μl of fresh DMEM complete medium.

⑥ After 96 hours, observe the fluorescence performance. The number of fluorescent cells shall decrease with increasing dilution concentration. Count the number of fluorescent cells (N) in the last well containing fluorescent cells. N divided by the corresponding dilution factor gives the titer value of the viral stock.

E.g. 5 fluorescent cells are counted from the well "10^{-5}" , then the viral titer of the viral stock is $5/10^{-5}=5\times10^5$TU/μL which is 5×10^8TU/ mL.

FAQ

1. Why 293T is chosen for lentivirus packaging, can other cells be used?

Because the transfection rate of 293T cells is high and the growth rate is fast, it is beneficial to improve the yield of virus. And 293T cells have been modified to allow packaging plasmids to exist in the cell for a longer period of time, it is beneficial to the expression of virus packaging elements and increase the virus yield. Also when selecting 293T cells, it is recommended to select 293T packaging cells from Ubigene. Because Ubigene' s 293T cells have STR authentification, and also have low passages, strong viability, rapid growth ratenot, strong culture survival ability as well as high transfectability. The virus yield is higher than common 293T cells, which can further improve the virus

packaging as well as the success rate of the subsequent cell transduction experiment.

2. Which components are included in the lentiviral packaging plasmid mix (LV PacMix)?

The lentivirus packaging plasmid mix (LV PacMix) provided in this kit contains three lentivirus packaging helper plasmids, which are pMDLG/pRRE, pCMV-VSVG, pRSV-REV. To further improve the virus yield, Ubigene has completely optimized the plasmid ratio, and the experimental data show that using this ratio can effectively improve the virus yield and achieve better virus packaging efficiency.

3. Is it possible for virus packaging when the cell confluence is not within the 80-90% range?

① It is possible for virus packaging when cell confluence below 80%, but it will affect virus yield and too low cell confluence also results in a poor cell condition during virus packaging.

② If cell confluence is higher than 90%, it will affect the transfection rate of the cells and also the virus yield, and at the high confluence the cell condition becomes unwell or the cells will be sloughed off.

4. How is virus packaging successful?

If the expression plasmid is fluorescent, this can be judged by observing the fluorescence performance and, in addition, by looking at the cells for lesions to see whether the virus has been packaged successfully. (there is a cell fusion phenomenon)

5. How long can the virus be stored below -70℃?

It is recommended for storage for no longer than 6 months.

6. Does repeated cryopreservation and thawing have an effect on the titer of virus?

Repeated cryopreservation and thawing will decrease the viral titer. Try to use up the virus at one time after thawing the virus. If there is any leftover, return to -70℃ for storage as soon as possible.

7. What is the function of Polybrene in the kit?

It is an infection reagent with a common concentration of 5~8 μg/ml. Polybrene can enhance the combination of lentivirus and cell membrane by neutralizing the interaction between charges, to improve the transduction efficiency of virus. However, Polybrene is toxic to some cells, and different cells have different sensitivity to Polybrene. If necessary, several working concentrations can be set to test the toxicity of Polybrene to target cells. The concentration of Polybrene provided by Ubigene is 0.5 mg/ml. If necessary, it can be diluted with PBS or culture medium during use.