

EZ-LentiPACK™ Packaging System (Cat. PAC-10001-20, PAC-10001-60)

Important Guidelines

EZ--LentiPACK is an optimized and ready--to use packaging system for the production of the highest titers of recombinant lentivirus.

Lentiviral packaging is the most critical step to generate high titer of lentiviral vectors. Several factors are crucial for the successful lentiviral vector production. Packaging plasmids and transfection reagent are the two most important factors among them. It is impossible to produce high quality virus without a good packaging plasmid. Impurity in the plasmid preparation results in incorrect DNA concentration in the packaging system and unhealthy packaging cells.

Two critical components for lentiviral vector packaging system are included in EZ--LentiPACK: the lentiviral packaging plasmid and the transfection reagent. The packaging plasmid included is the highest--grade plasmid in optimized ratio for lentiviral vector packaging. The second component is the transfection reagent ideal for lentiviral vector production, featuring high transfection efficiency and low toxicity to packaging cell line. A positive control of pLV--CE--CMV--GFP (10µg) is also included in the kit for quality control purpose.

Quality Control

Each batch of EZ--LentiPACK is tested for packaging performance and for the absence of DNA, RNA or microbial contamination.

Notices to all customers

All of the following steps should be performed in a sterile tissue culture hood. Lentivirus requires the use of a Biosafety Level 2 facility. VSV--G pseudotyped lentiviruses packaged from HIV--1--based vectors are capable of infecting human cells. Know and use appropriate safety precautions.

Protocol-Transfecting Adherent Mammalian Cells

Use the following procedure to produce lentiviral vector pseudotyped with VSV--G envelope. All amounts and volumes are given on a 100mm culture plate.

1. One day prior to the transfection, plate HEK293T cells in the complete growth medium without antibiotics. The cells should be 60--80% confluence at the time of transfection.
2. For each transfection sample, prepare complexes as follows

PACK Solution I:

EZ-LentiPACK™ Packaging System

Transfer Vector	8 µg
EZ-LentiPACK packaging plasmid	8 µg
Opti-MEM	1ml

PACK Solution II:

EZ-Transfx	18µl
Opti-MEM	1ml

- 1) Prepare PACK Solution I: dilute 8µg of your transfer vector DNA and 8µl of EZ--LentiPACK packaging plasmid with 1ml of Opti--MEM.
- 2) Prepare PACK Solution II: dilute 18µl of EZ--Transfx™ with 1ml of Opti--MEM.
3. Mix PACK Solution I and II, incubate the mixture at room temperature for 20 minutes.
4. Add 3ml of Opti--MEM to the mixture after the incubation.
5. Remove the culture medium from the cell dishes; add the mixture to the packaging cells. Incubate the cells at 37°C in a CO2 incubator for 6 hours (or overnight).
6. Replace with 10ml of fresh culture medium and continue culture cells in 37°C cell culture incubator with 5% CO2.
7. Harvest the lentiviral supernatants at 48 hours to 72 hours post transfection. Centrifuge briefly (500 x g for 10 min) to remove cellular debris.
8. Store at 4°C for short term or --80 °C for long term.