CELLOMICS TECHNOLOGY
Accelerate Life Science Research

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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 a high titer of lentiviral vectors. Several factors are crucial for the successful lentiviral vector production. Packaging plasmids and transfection reagents are the two most important factors among them. It is impossible to produce high quality viruses 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 the 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 lines. A positive control of pLV-CMV-GFP-PGK-Puro (10 µg) is also included in the kit for quality control purposes.

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.

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Protocol-Transfecting Adherent Mammalian Cells

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

Transfer Vector	8 µg
EZ-LentiPACK packaging plasmid	8 µg
EZ-Transfx Buffer	160 ul

PACK Solution II:

EZ-Transfx	16 µl
EZ-Transfx Buffer	160 ul

1. Prepare PACK Solution I: dilute 8 µg of your transfer vector DNA and 8 µg of EZ-LentiPACK packaging plasmid with 160 ul of EZ-Transfx Buffer, mix well by pipetting.

2. Prepare PACK Solution II: dilute 16 µl of EZ-Transfx with 160 ul of EX-Transfx Buffer, mix well by pipetting.

3. Mix PACK Solution I and II, pipetting up and down, you should see the solution getting cloudy, incubate the mixture at room temperature for 15 minutes.
Note: Mixing well is very important for efficient transfection, so is incubation time.

4. Add 4 ml of Opti-MEM to the mixture after the incubation, mix by pipetting.

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5. Remove the culture medium from the cell dishes; add the mixture to the packaging cells. Incubate the cells at 37°C in a CO_2 incubator for 1 hour to overnight .

6. Replace with 10 ml of fresh culture medium and continue culture cells in a 37°C cell culture incubator with 5% CO_2.

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.

To prevent lentivirus titer loss during storage and freeze-thaw cycles, it is recommended to add LentiGuard® to your lentivirus stock before snap-freeze.

For detailed information, please visit: www.cellomicstech.com and check lentiGuard.