Lenti-vpak Lentiviral Packaging Kit

Application Guide

Package Contents and Storage Conditions

Component	Amount	Storage Condition	Shipping Condition
Packaging Plasmids - lyophilized	60 µg	+4°C	On ice
TurboFectin Transfection Reagent - liquid	400 µL	+4°C	On ice

Content Reconstitution

Packaging Plasmids - Add 120 µL of distilled sterilized water (0.5 µg/µL). Please store at -20°C.

Reagents Required but Not Provided

HEK 293T Cells (ATCC)

Opti-MEM (ThermoFisher)

0.45µm PES filter

Related Products

shRNA Lenti: Kit includes 4 gene-specific constructs and a negative scrambled control.
Lenti-ORF Clones: Offered in 4 lenti vectors
Lentivirus concentrator: Concentrate up to 100 times
Lentivirus stablizer: Preserves lentiviral infectivity

Precautions and Disclaimers

These products are for R&D use only, and not for drug, household, or other uses.
Please consult the Material Safety Data Sheet (MSDS) for information regarding hazards and safe handling practices. Although the lentiviral transduction particles produced are replication incompetent, it is highly recommended that they be treated as Risk Group Level 2 (RGL-2) organisms. Follow all published RGL-2 guidelines for handling and waste decontamination.

Vessel	Cells	Lenti Plasmids	Packaging Plasmids	Transfection Reagent	Opti-MEM	Reactions per Kit
10-cm dish	$2.5x10^6$	5 µg	6 µg	33 µL	1.5 mL	10
6-well plate	$5x10^5$	1 µg	1.2 µg	6.6 µL	250 µL	50
12-well plate	$2.5x10^5$	0.5 µg	0.6 µg	3.3 µL	100 µL	100

OriGene Technologies, Inc.
9620 Medical Center Dr. Suite 200
Rockville, MD 20850
t: 888.267.4436
f: 301.340.8606
www.origene.com

Lenti-vpak Packaging Kit Protocol

*For OriGene's Lenti shRNA application, we recommend a 6-well plate format. Please refer to the table above.

**Protocol below is a for a 10cm dish, see the table above for different vessels and reagent requirements.

Day 1. Plate 2.5 x 10^6 of 293T cells on a 10cm dish in 10 mL complete growth media (antibiotic-free preferred) and incubate at 37°C overnight.

Day 2. Transfection

1) In a labeled Eppendorf tube, dilute the following DNA in 1.5 mL Opti-MEM, and pipet gently to mix completely.
   a. 5 µg of pLenti-shRNA construct or
      5 µg of pLenti-ORF expression construct
   b. 6 µg of packaging plasmids

2) Add 33 µL of TurboFectin transfection reagent to the diluted DNA (not the reversed order), pipet gently to mix completely.

3) Incubate for 15 min at room temperature.

4) Add the transfection mixture prepared above dropwise to the cells. Gently rock the plate back-and-forth and from side-to-side to distribute the complex evenly. Incubate at 37°.

Note: With TurboFectin, no medium change is necessary, directly add the transfection mixture to cells in complete growth media.

Day 3. Change the culture medium after 12-18 hours of incubation.

Day 4. Harvest the first batch of viral supernatant from the culture and store at 4°C.
Add 10 mL fresh culture media to the cell culture.

Day 5. Harvest the second batch of viral supernatant and combine with the first batch.

Filter through a 0.45µm PES filter to remove cellular debris.

The viral titer at this step is usually 10^6-10^7 TU/mL**. The viral supernatant is now ready for the majority of transduction applications. If necessary, further concentration can be applied.

** Large ORF inserts might decrease the viral titer

OriGene Technologies, Inc
9620 Medical Center Drive, Suite 200
Rockville, MD 20850
t: 888.267.4436
f: 301.340.8606
www.origene.com