

Lenti-vpak Lentiviral Packaging Kit

Application Guide

Cat# TR30040, TR30040P5 (5 X TR30040)

Package Contents and Storage Conditions

Component	Amount	Storage Condition	Shipping Condition
Packaging Plasmids - lyophilized	60 µg	+4°C	Room Temp
MegaTran 2.0 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 (Thermo Fisher)
 0.22 µm 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

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	6 µg	6 µg	40 µL	1.2 mL	10
6-well plate	5x10 ⁵	1 µg	1 µg	6.6 µL	200 µL	50
12-well plate	2.5x10 ⁵	0.5 µg	0.5 µg	3.3 µL	100 µL	100

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 10-cm dish, see the table above for different vessels and reagent requirements.

Day 1. Plate 2.5×10^6 of 293T cells on a 10-cm 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 0.6 mL Opti-MEM, and pipet gently to mix completely.
 - a. 6 µg of pLenti -shRNA construct or
6 µg of pLenti -ORF expression construct
 - b. 6 µg of packaging plasmids
- 2) Add 40 µL of MegaTran 2.0 transfection reagent to 0.6 mL Opti-MEM and vortex shortly, then add it to 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°.

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 0.22 µm 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 majority of transduction applications. If necessary, further concentration can be applied.

** Large ORF inserts might decrease the viral titer