

# 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              | Room Temp          |
| <a href="#">TurboFectin</a> 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 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.

### Reagent Requirements by Vessel Type

| Vessel        | Cells               | Lenti Plasmids | Packaging Plasmids | Transfection Reagent | Opti-MEM | Reactions per Kit |
|---------------|---------------------|----------------|--------------------|----------------------|----------|-------------------|
| 10-cm dish    | 2.5x10 <sup>6</sup> | 5 µg           | 6 µg               | 33 µL                | 1.5 mL   | 10                |
| 6-well plate  | 5x10 <sup>5</sup>   | 1 µg           | 1.2 µg             | 6.6 µL               | 250 µL   | 50                |
| 12-well plate | 2.5x10 <sup>5</sup> | 0.5 µg         | 0.6 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 10cm dish, see the table above for different vessels and reagent requirements.

Day 1. Plate  $2.5 \times 10^6$  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 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.