Lenti-vpak Lentiviral Packaging Kit

Application Guide

Package Contents and Storage Conditions

Component	Amount	Storage Condition	Shipping Condition
Packaging Plasmids - lyophilized*	60 ug	-20°C	Room Temp
MegaTran1.0 Transfection Reagent - liquid	500 ul	4°C	Room Temp

*Packaging Plasmids are a 3rd generation mixture

Content Reconstitution

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

Reagents Required but Not Provided

HEK 293T Cells (ATCC)
Opti-MEM (Life Technologies)
0.45 micron filter

Related Products

shRNA Lenti Kits – pGFP-C-shLenti vector. Kit includes 4 gene-specific constructs and a negative scrambled control.
TrueORF Lenti Clones – pLenti ORF vectors

Precautions and Disclaimers

These products are for R&D use only, not for drug, household, or other uses.
Please consult the Material Safety Data Sheet 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	shRNA Plasmid	Packaging Plasmids	Transfection Reagent	Opti-MEM Per Vial	Reactions per Kit
10 cm dish	2.5x10^6	5 ug	6 ug	44 ul	500 ul	10
6-well plate	5x10^5	1 ug	1.2 ug	8.8 ul	100 ul	50
12-well plate	2.5x10^5	0.5 ug	0.6 ug	4.4 ul	50 ul	100

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Lenti-vpak Packaging Kit Protocol

*For OriGene's Lenti shRNA application, we recommend a 6-well plate format, please see 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 and incubate at 37^{o}C overnight.

Day 2. Transfection,

1) In a labeled ependorf tube (vial 1), mix the following DNA with 500ul Opti-MEM
   a. 5 ug of pLenti-shRNA construct or
      5 ug of pLenti-ORF expression construct
   b. 6 ug of packaging plasmids
2) In a separate tube (vial 2), mix 44ul of MegaTran transfection reagent with 500ul Opti-MEM.
3) Transfer the DNA solution from vial 1 into vial 2 containing MegaTran. Vortex it and incubate 15-30 min at room temperature.
4) Add the mixture of DNA and MegaTran directly to the 10cm dish of 293T cells.

Day 3. After 12-18 hrs incubation, change the culture medium.

Day 4. Harvest the first batch of viral supernatant from the culture and store it at 4^{o}C. Add fresh culture medium to the cell culture.

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

Spin 3000rpm/min and filter through a 0.45 micron 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 will decrease the viral titer

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rev02/2013