

Lentiviral Packaging Mix Specification Sheet

Product Name	Lentiviral Packaging Mix
Description	<p>For the production of lentiviral particles, three components are generally required: 1) a lentiviral vector containing your inserts of interests, 2) packaging vectors containing all necessary viral structure proteins, 3) an envelope vector expressing Vesicular Stomatitis Virus (VSV) glycoprotein (G). The third generation packaging system offers maximal biosafety as the lentiviral Rev gene is supplied as an independent vector from other structure genes, further eliminating the possibility of reverse recombination of vectors into a replication competent viral particle. The third generation lentiviral packaging mix will only support lentiviral expression vector with a chimeric 5' LTR in which the HIV promoter is replaced with CMV or RSV, thus making it TAT-independent.</p> <p>SuperLenti™ Lentivirus packaging mix is a ready-to-use third generation HIV-based lentiviral packaging system in which the plasmids express the elements required for lentiviral production, which allows creation of a replication-incompetent, HIV-1-based lentivirus that is used to deliver and express your gene of interest in either dividing or non-dividing mammalian cells.</p>
Catalog Number	VP100
Size	200 µl
Shipping	Ambient temperature
Storage and Stability	Store at -20 °C. This product is stable for 6 months when stored as directed. Freeze-thaw cycles should be minimized by dividing into single-use aliquots. Store aliquots in the -20 °C freezer until ready for use.
Quality Control	Each lot of lentiviral packaging mix is functionally tested in transfection assays using human embryonic kidney 293 cells.
Usage	<p>For 100 mm dish of lentiviral packaging, mix 2.5 µg of lentiviral expression vector with 20 µl of lentiviral packaging mix.</p> <p>For 150 mm dish of lentiviral packaging, mix 5 µg of lentiviral expression vector with 40 µl lentiviral packaging mix.</p>
Restricted Use	For Research Use Only. Not for use in diagnostic or therapeutic procedures.

Applications:



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The Lentiviral Packaging Mix is an optimized formulation of third generation of packaging plasmids that supply the helper functions as well as structural and replication proteins that are required to produce high-titer lentivirus.

Protocol

SuperLenti™ Lentivirus Packaging Mix Cat. no. VP100

Condition	Quantity
Tissue culture plate size	10 cm (one per lentiviral vector)
Number of 293FT cells to transfect	4×10^6 cells
Amount of Lentivirus Packaging Mix	20 μ l
Amount of pLenti expression vector	2.5 μ g
Amount of NanoFect (Cat.no. NF100)	35 μ l
Amount of serum free DMEM	1 ml

Procedure

DAY 1:

1. Seed $\sim 4 \times 10^6$ HEK 293T cells into a 100-mm dish.

DAY 2:

2. Check to make sure the cells are 70-90% confluent.
3. For each 100 mm dish prepare transfection complex as follows:
 - a. 1.5ml Tube A: Dilute 2.5 μ g DNA plasmids and 20 μ l Lentivirus Packaging Mix in 0.5ml DMEM or Opti-MEM I Medium without serum and mix gently.
 - b. 1.5ml Tube B: Dilute 35 μ l of NanoFect transfection reagent (ALSTEM, cat.no. NF100) in 0.5 ml DMEM or Opti-MEM I Medium without serum and mix gently.
 - c. Add NanoFect/DMEM in Tube B into DNA/DMEM solution (Tube A). Vortex for 5-10 seconds and incubate the DMEM-plasmid-NanoFect mixture at room temperature for 15 minutes.
4. Add the complete transfection complex from step 3 dropwise to the plate of cells, and rock the plate back and forth to disperse the transfection complex evenly in the plate.
5. Incubate the cells overnight at 37°C in a humidified 5% CO₂ incubator.

DAY 3:

6. Replace supernatant with 10 ml fresh media.

Note: You may supplement the culture medium with 20 μ l of ViralBoost (500X, ALSTEM, cat.no. VB100) to enhance the virus titer.



7. Incubate at 37°C for 24 hours.

DAY 4:

8. Collect supernatant medium that contains lentivirus into a 50ml sterile, capped conical centrifuge tube and put on ice.

9. Centrifuge supernatant at 3000 rpm for 15 minutes at 4°C to pellet cell debris.

10. Filter the cleared supernatant with a low-protein binding 0.45µm sterile filter.

11. The virus is ready for infection, purification, or it can be stored as a viral stock at -80°C for your future applications. Aliquot volumes are preferred for storage to reduce the viral titer loss from freeze-thaw cycles.

***Note:** Expression of the VSVG glycoprotein causes HEK 293T cells to fuse, resulting in the appearance of large, multinucleated cells known as syncytia. This morphological change is normal and does not affect the production of the lentivirus.*

