

Product Specification Sheet

Product Name	Lentivirus 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, and 3) an envelope vector expressing Vesicular Stomatitis Virus glycoprotein (VSVG). 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. 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.
Usage	<p>For 100 mm dish of lentiviral packaging, mix 2.5 µg of lentiviral expression vector with 20 µl of lentivirus 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>
Quality Control	Each lot of Lentivirus packaging mix is functionally tested in transfection assays using human embryonic kidney 293 cells.
Restricted Use	For Research Use Only. Not for use in diagnostic or therapeutic procedures.



Protocol (VP100)

Materials Needed

Tissue culture plate	10 cm (one per lentiviral vector)
293FT cells to transfect	4 x 10 ⁶ cells
Lentivirus Packaging Mix	20 µl
pLenti expression vector	2.5 µg
NanoFect™ (Cat. # NF100)	30 µl
Serum-free DMEM	1 ml

Procedure

Day 1

1. Seed ~4 x 10⁶ 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. 1.5 ml Tube A: Dilute 2.5 µg DNA plasmids and 20 µl Lentivirus Packaging Mix in 0.5 ml DMEM or Opti-MEM I Medium without serum and mix gently.
 - b. 1.5 ml Tube B: Dilute 30 µL of NanoFect™ 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 sec and incubate the DMEM-plasmid-NanoFect™ mixture at room temperature for 15 min.
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 and supplement with 20 µl of ViralBoost™ (Cat. # VB100).
7. Incubate at 37° C for 24 hours.



Day 4

8. Collect supernatant medium that contains lentivirus into a 50 ml 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 0.45 µm 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.





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