Lentiviral Packaging Mix Protocol

Catalogue number: VP100

Product Descriptions

Lentiviral 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. To produce high quality lentiviral particles, all you need is a lentiviral expression vector containing your gene of interest. Lentiviral Packaging Mix contains the essential packaging vectors containing the viral structure proteins and the envelope vector to express the Vesicular Stomatitis Virus glycoprotein (VSVG). The lentiviral packaging mix is TAT-independent because it only supports lentiviral expression vector with a chimeric 5' LTR, in which the HIV promoter is replaced with CMV or RSV.

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 µl
Amount of pLenti expression vector	2.5 µg
Amount of NanoFect (Cat.no. NF100)	35 µl
Amount of serum free DMEM	1 ml

Procedure

DAY 1:
1. Seed ~4 X 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 µg DNA plasmids and 20µl Lentivirus Packaging Mix in 0.5ml DMEM or Opti-MEM I Medium without serum and mix gently.
b. 1.5ml Tube B: Dilute 35 µL 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.

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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% CO_2 incubator.

DAY 3:
6. Replace supernatant with 10 ml fresh media.

> ***Note:*** *You may supplement the culture medium with 20 µl 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.*

Related Products

Products	Cat #	Description
ViralBoost Reagent	VB100	A novel cocktail of small molecules that can enhance viral production.
Virus Protection Medium	VF010	Preserve functional viral particles during repetitive freeze-thaw cycles.
TransPlus™ Virus Transduction Enhancer	V020	Mixture of polymers optimized for the infection of lentivirus or retrovirus to most cells.
NanoFect Transfection Reagent	NF100	Nanotechnology-based reagent providing efficient gene delivery for most cell types.
Lentivirus Precipitation Solution	VC100	Concentrate Lentiviral particles up to 100 fold in 4 hours.
Lentiviral Expression Vectors	LV series	Various lentiviral expression vectors for your research needs.
Lentiviral Reporter Plasmids	LR series	Various lentiviral reporter vectors for your research needs.