Retroviral Packaging Mix Protocol

Catalogue number: VP200

Product Descriptions

The Retroviral Packaging Mix provides a rapid and convenient method for producing high titers of replication-incompetent retroviruses. This Moloney Murine Leukemia Virus (MMLV)-based packaging system possesses the ideal host range required for your target cells. It is a ready-to-use retroviral packaging mix, which contains VSVG and produces high titer retroviruses in only 48 hours. Separate expression of these essential viral structural genes ensures safety and control over the recombinant constructs by minimizing the risks that arise during cell division and reducing the chance of producing replication-competent viruses.

Protocol

Condition	Quantity
Tissue culture plate size	10 cm (one per lentiviral vector)
Number of 293T cells to transfect	4 × 106 cells
Amount of retroviral packaging mix	15 µl
Amount of pRetro expression vector	7.5 µg
Amount of NanoFect (Cat.no. NF100)	45 µl
Amount of serum free DMEM	500 µl

DAY 1:

1. Seed 293T cells in 10cm dishes (~4 X 106 cells per 10cm dish).

DAY 2:

2. Check to make sure the cells are 70-80% confluent.

3. For each 10cm dish prepare transfection complex as follows:

a. 1.5ml Tube A: Dilute 7.5 µg DNA plasmid and 15 µl retroviral packaging mix in 0.5 ml serum-free medium (e.g. DMEM). Mix by pipetting.

b. 1.5ml Tube B: Dilute 45 µL of NanoFectTM transfection reagent in 0.5 ml serum-free medium and mix gently.

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c. Add NanoFect/DMEM into DNA/DMEM solution. Mix by vortexing for 5-10 seconds and then incubate the DMEM-plasmid-NanoFect mixture at room temperature for 15 minutes.

4. Add the complete transfection complex from step 3 drop-wise to the cells, and swirl the dish to disperse the transfection complex evenly in the plate.

5. Incubate the plate at 37°C overnight.

DAY 3:

6. Replace the supernatant with 10 ml fresh media.

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

7. Incubate at 37°C for 24 hours.

DAY 4(1st Harvest):

8. Collect the supernatant that contains retroviruses into a 50ml sterile, capped conical centrifuge tube.

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

10. Filter the clear supernatant through 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.

12. If second harvest is needed, add 10 ml of complete medium to the cells after the first harvest and put the dish back to 37°C incubator.

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

DAY 5(2nd Harvest)

13. The second harvest can be done on Day 5, following steps 8-11.

14. To concentrate virus, add a 1 volume Retrovirus Concentration Solution (ALSTEM, cat.no.VC200) to 4 volumes of the viral supernatant, and mix thoroughly.

15. Put the mixture to 4°C refrigerator overnight and pellet the viruses next day. Please refer the user manual for details.

Related Products

Products	Catalogue number	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	NF 100	Nanotechnology-based reagent providing efficient gene delivery for most cell types.
Retrovirus Precipitation Solution	VC200	Concentrate retroviral particles up to 100 fold in 4 hours.