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Lentivirus Transfection Reagen

1 Components

Components	HY-K2015-1 mL	HY-K2015-5 mL
Lentivirus Transfection Reagent	1 mL	1 mL × 5

2 Introduction

Lentivirus vector is a gene therapy vector based on HIV-1, which can stably integrate exogenous genes into the host cell genome. It offers the advantages such as an extensive infection spectrum, effective infection and division period, static cell cells, long-term stable expression of exogenous genes, etc. It has become a powerful tool for importing exogenous genes, and has been widely used in gene overexpression in various cell lines, RNA interference, microRNA research and in vivo animal experiments.

MCE Lentivirus Transfection Reagent is a new type of transfection reagent based on cationic polymer, which is suitable for lentivirus packaging and transfection. It significantly improves the efficiency of packaging and transfection, resulting in the production of more recombinant lentivirus. The viral titer of the control plasmid obtained by transfection and packaging can reach 10^8 TU/mL, which has the advantages of high infection efficiency, wide infection spectrum, high reproducibility and simple operation.

3 Components

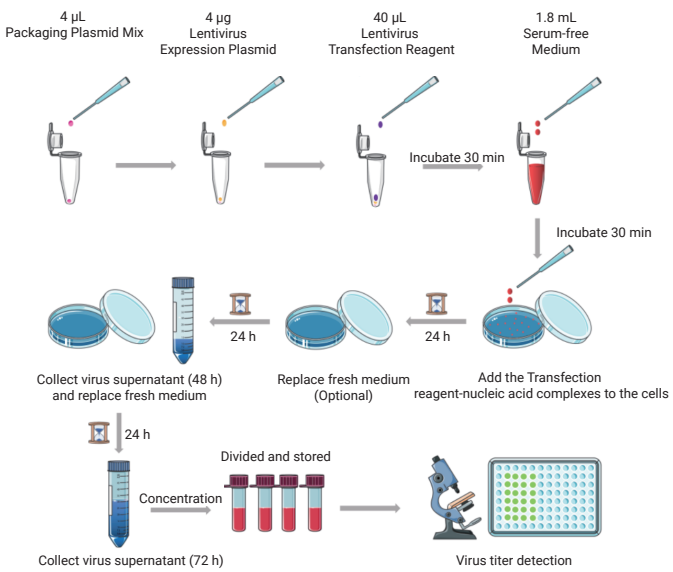

Figure 1. The process of the Lentivirus Packaging Transfection

4 General Protocol

This is the protocol for transfection in the 100 mm dish, and the adding volume of other culture devices is shown in Table 1.

1.Prepare cells

24 h prior to transfection, plate 4-6 × 10^6 of cells (293 or 293T, etc.) on a 10cm dish and incubate until the density reaches 70%-80% for cell transfection.

2.Lentivirus packaging

(1)Take 4 μL of Lentivirus packaging aid plasmid mixture into a 2 mL centrifuge tube, add 4 μg of lentiviral vector plasmid containing the target gene, mix gently, add 40 μL of MCE Lentivirus Transfection Reagent and mix gently, incubate for 3 min at room temperature.

(2)Add 1.8 mL of serum-free basal medium, mix gently and incubate for 30 minutes at room temperature.

Note: Transfection reagent-nucleic acid complex is stable for 4 h at room temperature.

(3)Add the Transfection reagent-nucleic acid complexes (1.8mL) to the cells and mix gently, and incubator for further culture.

Note: Take care to be slow in dripping to avoid washing up the cells and to mix gently.

3.Harvesting of Lentivirus

(1)After transfection for 24 h, discard the initial viral supernatant, add 10-15 mL of fresh complete medium (including serum), and continue incubating for further culture.

(2)After transfection for 48 h, collect the viral supernatant, add 10-15 mL of fresh complete medium (including serum), and continue incubating further culture.

(3)After transfection for 72 h, observe the cell state and take pictures. Collect the viral supernatant, mix with the supernatant collected by 48 h. Centrifuge the mixture at 800 × g for 10 min to remove cell debris, then filter it using a 0.45 μm filter. The filtered superntant can be used to directly infect the cells or concentrated to obtain a lentiviral concentrate with higher titer.

Note: The titer of the virus will be reduced by 10%-20% within a freeze-thaw. Lentivirus is recommended to be stored at -80°C after dispensing and used within six months.

Table 1 PEI Transfection Reagent: DNA Ratio

Plate Size	Growth Medium (mL)	DNA (μg)	Lentivirus Transfection Reagent (μL)	Serum-freeMedium (μL)
6-well	2	2	8	400
60 mm	4	4	8-16	800
100 mm	10	5-10	20-40	1800
125 mL	30-35	30-35	60-120	6000
500 mL	120-140	120-140	250-500	25000
1000 mL	240-280	240-280	500-1000	50000

4.Analysis of lentivirus titer assays

Using the gradient dilution method to infect cells, and viral titers were determined by PCR, qPCR, etc.

5 Storage

-20°C，1 year

Avoid repeat freeze-thaw cycles.

6 Precautions

1.The viability and general health of cells prior to transfection significantly affect the transfection result.

For transfection, it is recommended to use high-quality plasmids that are endotoxin-free, high purity, contamination-free and sterile.

2.The amount of plasmid should be reasonably calculated, transfection of too much plasmid may lead to cell death.

3.Cell status affects the transfection efficiency and thus the quality of lentivirus. It is recommended to use cells in the exponential phase of growth with a survival rate of more than 90% for lentiviral packaging. If the cells have just been resuscitated, it is better to passage the cells for two generations before packaging.

4.MCE Lentivirus Transfection Reagent can be used for transfection in serum-containing media and do not require media change.

5.To improve transfection efficiency, Serum-free Medium is recommended to prepare the transfection complex.

6.All operations should be carried out in a BSL2 biological safety cabinet.

7.This product is for R&D use only, not for drug, household, or other uses.

8.For your safety and health, please wear a lab coat and disposable gloves to operate.