LIFESCT
LIFE SCIENCE BIOTECHNOLOGY

Email: info@lifesct.com
Tel: +1-301-328-1977 Fax: +1-301-965-8269
9610 Medical Center Drive, Rockville, MD 20850, USA

LiFect293™ Transfection Reagent

Cat. #: M0002 Size: 1 ml/4 ml

Protocol for generation of Lentivirus from 293T cell

Introduction:

LiFect293™ is an enhanced liposome-based DNA transfection reagent which is specifically formulated and optimized for HEK293 cells and other mammalian cells with superior efficiency and invisible cytotoxicity. LiFect293™ Reagent, 1.0 ml, is sufficient for 300 to 600 transfections in 24 well plates or 50 to 100 transfections in 6 well plates.

Important Guidelines for Transfection:

- For high efficiency, transfect cells at high density. 80~90% confluency is highly recommended

- To lower cytotoxicity, transfect cells in presence of serum (10%) and antibiotics

- Change medium with serum (10%) and antibiotics 5 hours post transfection is optional

Procedures for Transfecting 293T Cells

Cell Seeding:

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal 80~90% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30~60 minutes before transfection. (see **Table 1**)

Note: High serum levels (>5%) with antibiotics usually do not have inhibitory effect on transfection efficiency. For some specific 293 cells, maximal transfection efficiencies are observed in the presence of serum and antibiotics. We recommend using complete serum/antibiotics-containing medium initially.

Preparation of LiFect293™/DNA Complex and Transfection Procedures:

The following protocol is given for transfection in 10 cm dish. For other culture formats, scale up or down per culture dish's surface. The optimal transfection conditions are given in the standard protocol described below.

- Cell confluency should be 80~90 % at the day of transfection

- For each 10 cm dish, add 7.0 ml of complete medium with serum and antibiotics freshly 30~60 min before transfection.

Table 1. A Guideline for Seeding Adherent Cells Prior to Transfection in Different Culture Formats.

Culture Dish	Surface Area (cm^2)	Number of Cells to Seed
T75 Flask	75	$3.0 - 6.0 \times 10^6$
100 mm dish	58	$2.2 - 4.4 \times 10^6$
60 mm dish	21	$0.9 - 1.8 \times 10^6$
35 mm dish	9.6	$3.5 - 7.0 \times 10^5$
6-well plate	9.6	$4.0 - 8.0 \times 10^5$
12-well plate	3.5	$1.5 - 3.0 \times 10^5$
24-well plate	1.9	$0.8 - 1.6 \times 10^5$
48-well plate	1.0	$4.0 - 8.0 \times 10^4$
96-well plate	0.3	$1.2 - 2.4 \times 10^4$

- For each dish, dilute total 10 µg of DNA (5.0 µg lenti-vector plasmid plus 5.0 µg lentivirus packaging mix) into 400 µL of serum-free DMEM with High Glucose. Vortex to mix.

- For each dish, dilute 30 µL of LiFect293™ reagent into 400 µL of serum-free DMEM with High Glucose. Vortex gently to mix.

Note: Never use Opti-MEM to dilute plasmid DNA and LiFect293™ reagent because it will disrupt transfection complex.

- Add the diluted LiFect293™ Reagent immediately to the diluted DNA solution all at once. (**Important**: do not mix the solutions in the reverse order !)

- Vortex to mix the solution immediately followed by incubation of 10 min at room temperature to allow LiFect293™/DNA complexes to form.

Note: Never keep the DNA/LiFect293™ complex longer than 20 min

- Add the 800 µL LiFect293™/DNA complex dropwise onto the medium in each dish and homogenize the mixture by gently swirling the plate.

- Check transfection efficiency and harvest lentivirus supernatant 24 to 72 hours post transfection.

Storage: LiFect293™ In Vitro DNA Transfection Reagent is stable for up to 12 months at 4°C.

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