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LiFect293™ Transfection Reagent

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

Protocol for generation of rAAV 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 150 mm 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 15 cm dish, add 15 ml of complete medium with serum and antibiotics freshly 30~60 minutes 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 × 10^6
100 mm dish	58	2.2 - 4.4 × 10^6
60 mm dish	21	0.9 - 1.8 × 10^6
35 mm dish	9.6	3.5 - 7.0 × 10^5
6-well plate	9.6	4.0 - 8.0 × 10^5
12-well plate	3.5	1.5 - 3.0 × 10^5
24-well plate	1.9	0.8 - 1.6 × 10^5
48-well plate	1.0	4.0 - 8.0 × 10^4
96-well plate	0.3	1.2 - 2.4 × 10^4

- For each dish, dilute 10 µg of rAAV cis plsamid, 10 µg capsid DNA and 16 µg helper DNA (total 36 µg DNA) into 750 µl serum-free DMEM with high glucose. Vortex briefly to mix.

- For each dish, dilute 100 µl of LiFect293™ reagent into 750 µl of serum-free DMEM with High Glucose. Vortex gently to mix.

Note: Never use Opti-MEM to dilute 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 !)

- Immediately pipette up and down 3~4 times or vortex briefly to mix followed by incubation for ~10 minutes at room temperature to allow DNA/LiFect293™ transfection complex to form.

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

- Add the 1000 µl LiFect293™/ DNA complex drop-wise onto the medium in each dish and homogenize the mixture by gently swirling the plate.

- Remove DNA/LiFect293™ complex-containing medium and replace with fresh complete serum-antibiotics containing medium ~5 hours post transfection.

- Check transfection efficiency and virus titer 24 to 48 hours post transfection. 48 hours gives better titers.

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

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