Cat # SL100668 Store at 4 °C

LipoD293™ In Vitro DNA Transfection Reagent (Ver. II)

----- A Protocol for generation of Lentivirus from 293T cell

- ☐ 100 µl
- ☐ 500 µl
- ☐ 1000 µl

SignaGen® Laboratories
9601 Medical Center Drive
A/R Bldg, Ste 341
Rockville MD 20850
FAX. 301-560-4919
TEL. 301-330-5966
Toll Free. 1-(866)-918-6812
Email: info@signagenlabs.com
Web: www.signagenlabs.com

This product is for laboratory research ONLY and not for diagnostic use

Introduction:

LipoD293™ (Ver. II) 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. LipoD293™ Reagent (Ver. II), 1.0 ml, is sufficient for 300 to 600 transfections in 24 well plates or 50 to 100 transfections in 6 well plates.

Important Transfection Guidelines:

- Do NOT follow transfection procedures for LipoD293 old version. Read protocol for new version carefully before transfection
- For high efficiency, transfect cells at high density. 90~95% 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 (see Table 1):

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

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.

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

Culture Dishes	Surface Area (cm2)	Number of Cells to Seed
T75 Flask	75	3.0 – 6.0 x 10^6
100 mm Dish	58	2.2 – 4.4 x 10^6
60 mm Dish	21	0.9 – 1.8 x 10^6
35 mm Dish	9.6	3.5 – 7.0 x 10^5
6-well Plate	9.6	4.0 – 8.0 x 10^5
12-well Plate	3.5	1.5 – 3.0 x 10^5
24-well Plate	1.9	0.8 – 1.6 x 10^5
48-well Plate	1.0	4.0 – 8.0 x 10^4
96-well Plate	0.3	1.2 – 2.4 x 10^4

Preparation of LipoD293™-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.
- 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 LipoD293™ 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 LipoD293™ reagent because it will disrupt transfection complex.

- Add the diluted LipoD293™ 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 LipoD293™-DNA complexes to form.

Note: Never keep the DNA/LipoD293™ complex longer than 20 min

- Add the 800 µL LipoD293™/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: LipoD293™ DNA In Vitro Transfection Reagent is stable for up to 12 months at +4 °C. This item shipped at ambient temperature