Cat # SL100668 Store at 4 °C

LipoD293™ DNA In Vitro Transfection Reagent

----- A Protocol for Transfections of Mammalian Cell

- ☐ 100 µl
- ☐ 500 µl
- ☐ 1000 µl

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

Introduction:

LipoD293™ DNA In Vitro Tranfection Reagent is a powerful transfection Reagent that ensures effective and reproducible transfection with invisible cytotoxicity. LipoD293™ is a specially designed cationic lipid formulation which offers extremely high transfection efficiencies of HEK293 cells as well as many mammalian cells. LipoD293™ Reagent, 1.0 ml, is sufficient for 300 to 600 transfections in 24 well plates or 50 to 100 transfections in 6 well plates.

Features:

- Invisible cytotoxicity
- Exceptional transfection efficiency on HEK293 cells
- Efficient transfection with or without serum
- High levels of recombinant protein production
- Simple, robust transfection procedure

Procedures for Transfecting Mammalian 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 60 70% confluency at the time of transfection. Serum-free DMEM medium is changed to replace complete serum-containing culture medium 30 minutes before transfection.

Note: High serum levels (>5%) have a moderate inhibitory effect on LipoD293™-mediated transfections. Maximal transfection efficiencies are observed in the absence of serum. Depending upon the cell type, the presence of serum <5% may sometimes improve the overall levels of recombinant protein expression.

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
T175 Flask	175	0.7–1.4 x 10^7
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 optimal ratio of LipoD293™/DNA is of 3/1. We recommend using serum-free DMEM with High Glucose to dilute DNA and LipoD293™ Reagent to ensure the optimal size of complex particles.

The following protocol is given for transfection in 24-well plates, refer to **Table 2** for transfection in other culture formats. The optimal transfection conditions for HEK293 cell are given in the standard protocol described below.

- For each well, dilute 1 µg of DNA into 50 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.
- For each well, dilute 3 µl of LipoD293™ solution into 50 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.
- Add the 50 µl LipoD293™ solution to the 50 µl DNA solution all at once. (**Important: do not mix the solutions in the reverse order !**)
- Vortex- mix the solution immediately and spin down briefly to bring drops to the bottom of the tube.
- Incubate for 10 minutes at room temperature.
- Add the 100 µl LipoD293™/ DNA mixture drop-wise onto the medium in each well and homogenize the mixture by gently swirling the plate.
- For maximal transfection efficiency, change the medium to complete serum containing medium 4~5 hours post addition of LipoD293™/DNA complex.
- Check transfection efficiency24 to 48 hours post transfection.

Table 2. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Culture Volume (ml)	Plasmid DNA (µg)	Diluent Volume (mL)	GenJet™ Reagent (µL)
6-well plate	1.6	2 - 3	0.20	6 - 9
35 mm dish	1.6	2 - 3	0.20	6 - 9
60 mm dish	4.5	5	0.50	15
100 mm dish	8	7 - 8	1.0	21 - 24
T75 flask	15	18 - 36	1.5	54 - 108
250 ml flask	50	50 - 100	2.5	150 - 300

Storage: LipoD293™ DAN In Vitro Transfection Reagent is stable for up to 18 months at 4 °C. This item shipped at ambient temperature