Cat # SL100668 Store at 4 °C

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

----- A General Protocol for transfecting Mammalian Cells

- ☐ 100 µl
- ☐ 500 µl
- ☐ 1000 µl

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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 mammalian cells with superior efficiency and less cytotoxicity. LipoD293™ Reagent (Ver. II), 1.0 ml, is sufficient for 600 to 1200 transfections in 24 well plates or 300 to 600 transfections in 6 well plates.

Important Guidelines for Transfection:

- LipoD293™ reagent was formulated for DNA transfection ONLY! The following standard protocols are for transfecting mammalian cells. The protocols for lentivirus production and insect cell transfection can be downloaded from our website
- For better efficiency, choosing a correct protocol is essential. We strongly encourage to use "General Protocol" first. If the "General Protocol" fails to give satisfactory result (e.g., less than 10%), try the "Advanced Protocol" in the back page
- For high efficiency and lower toxicity, transfect cells at high density. 70~80% confluency is highly recommended
- To lower cytotoxicity, transfect cells in presence of serum (10%) and antibiotics

Part I. General Procedures for Transfecting Mammalian Cells

Step I. Cell Seeding:

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal 70~80% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30~60 minutes before transfection.

Note: For high efficiency and lower toxicity, high cell density (~80% confluency) is essential. High serum levels (>5%) usually do not have inhibitory effect on transfection efficiency.

Table 1. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Volume (ml)	Plasmid DNA (µg)	Diluent Volume (mL)	LipoD293™ Reagent (µL)
48 well plate	0.3	0.25	2 x 0.015	0.75
12 well plate	0.75	0.75	2 x 0.038	2.25
6-well/35 mm dish	1.0	1	2 x 0.05	3.0
60 mm dish	2.8	2.5	2 x 0.10	7.5
10 cm dish	5.0	3 - 4	2 x 0.25	9 - 12
T75 flask	8.0	9 - 18	2 x 0.40	27 - 54
250 ml flask	18	25 - 35	2 x 0.8	75 - 105

Step II. Preparation of LipoD293™-DNA Complex and Transfection Procedures

For different cell types, the optimal ratio of LipoD293™ (µL):DNA (µg) is around 3:1. We recommend the LipoD293™ (µL):DNA (µg) ratio of 3:1 as a starting point which usually gives satisfactory transfection efficiency with invisible cytotoxicity. To ensure the optimal size of complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and LipoD293™ Reagent.

The following protocol is given for transfection in 24-well plates, refer to **Table 1** for transfection in other culture formats. The optimal transfection conditions for a majority of adherent cell lines are given in the standard protocol described below.

- For each well, add 0.5 ml of complete medium with serum and antibiotics freshly 30~60 minutes before transfection.
- For each well, dilute 0.5 µg of DNA into 25 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.
- For each well, dilute 1.5 µl of LipoD293™ reagent into 25 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.

  Note: Never use Opti-MEM to dilute LipoD293™ reagent and DNA, 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 !**)
- Immediately pipette up and down 3~4 times or vortex briefly to mix followed by incubation for ~15 minutes at room temperature to allow DNA-LipoD293™ transfection complexes to form.

  Note: Never keep the DNA/LipoD293™ complex longer than 30 minutes
- Add the 50 µl LipoD293™/ DNA mixture drop-wise onto the medium in each well and homogenize the mixture by gently swirling the plate.
- Remove LipoD293™/DNA complex-containing medium and replace with complete serum/antibiotics containing medium 12~18 hours post transfection. **For sensitive cells, to lower cytotoxicity, remove LipoD293 -DNA complex and replace with complete medium 5 hours after transfection.**
- Check transfection efficiency 24 to 48 hours post transfection.