

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

----- An Advanced Protocol for Transfecting Hard-to-transfect Mammalian Cells

- 100 µl
- 500 µl
- 1000 µl



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

Part II. Advanced Protocol for Transfecting Hard-To-Transfect Mammalian Cells

Important: The advanced protocol for hard-to-transfect cells is provided only if general protocol fails to give satisfactory efficiency (e.g., less than 10% efficiency). **For transfecting primary cells which cannot be trypsinized, go directly to Step II, skip trypsinization and incubate freshly prepared primary cell pellet with transfection complex.**

Step I. Cell Culture Before Transfection

Cells should be plated at least 24 hours prior to transfection so that the monolayer cell density reaches to the optimal 95~100% confluency at the day of transfection.

Table 2. A Guideline for Optimal Cell Number Per Well in Different Culture Formats

Culture Dishes	Surface Area (cm ²)	Optimal Cell Number
T75 Flask	75	9.6 x 10 ⁶
100 mm Dish	58	7.3 x 10 ⁶
60 mm Dish	21	2.7 x 10 ⁶
35 mm Dish	9.6	1.2 x 10 ⁶
6-well Plate	9.6	1.2 x 10 ⁶
12-well Plate	3.5	0.44 x 10 ⁶
24-well Plate	1.9	0.24 x 10 ⁶
48-well Plate	1.0	0.11 x 10 ⁶
96-well Plate	0.3	0.31 x 10 ⁵

Table 3. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Transfection Complex Volume (ml)	Plasmid DNA (µg)	LipoD293™ Reagent (µL)
96-well	0.02	0.2	0.8
48-well	0.04	0.5	2
24-well	0.1	1.0	4
6-well	0.2	2	8
35 mm dish	0.2	2	8
60 mm dish	0.5	5	20
10 cm dish	1.0	8	32
T75 flask	1.5	36	144

Step II. Preparation of Cells in Suspension

The following protocol is given for transfecting hard-to-transfect mammalian cells in 6-well plates, refer to [Table 2](#) for optimal cell

number per well per culture vessels' surface area. The optimal transfection conditions for mammalian cells are given in the standard protocol described below.

- Detach the cells with trypsin/EDTA and stop the trypsinization with complete culture medium.

Note: Cells that are difficult to detach may be placed at 37 °C for 5~15 min to facilitate detachment

- Take an aliquot of trypsinized cell suspension and count the cells to determine the cell density.
- Centrifuge the required 1.2x10⁶ cells per well for 6-well plate at 150xg at room temperature for 10 min.
- Use fine tip pipette to remove supernatant **completely** so that no residual medium covers the cell pellet.

Step III. Preparation and Application of Transfection Complex

For hard-to-transfect mammalian cells, the optimal ratio of LipoD293™ (µL):DNA (µg) is 4:1. 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 6-well plates, refer to [Table 3](#) for transfection in other culture formats.

- For each well of 6-well plate, dilute 2 µg of DNA into 100 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly to bring drops to bottom of the tube.
- For each well of 6-well plate, dilute 8 µl of LipoD293™ reagent (Ver. II) into 100 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.
- Add the diluted LipoD293™ Reagent immediately to the diluted DNA solution all at once.
- Vortex-mix the solution immediately and spin down briefly to bring drops to bottom of the tube followed by incubation of 10 minutes at room temperature to allow transfection complexes to form. **Important:** Never keep the transfection complexes longer than 15 minutes
- **Gently** resuspend the cell pellet prepared from [Step II](#) immediately in the 200 µl transfection complex and incubate at 37 °C for 15 minutes.
- At the end of incubation, add 2.0 ml of pre-warmed fresh complete cell growth medium to cells and plate onto one well of a 6-well plate.
- Remove transfection complex containing medium gently and refill with complete culture medium 12~18 hours after plating.
- Check efficiency 24~48 hours post transfection.