Cat # SL100668 Store at 4 °C

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

-----A Protocol for Transfecting 293 and CHO Cells in Suspension

- ☐ 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 HEK293 cells and other mammalian cells with superior efficiency and less cytotoxicity.

Important Guidelines for Transfection:

- LipoD293™ reagent was formulated for DNA transfection ONLY! The following standard protocol is for transfecting suspension 293 or CHO cells.
- To request protocol for lentivirus production and insect cells transfection, please email us at info@signagen.com or visit our website at www.signagen.com

Procedures for Transfecting Suspension 293 or CHO Cells

Follow the procedure below to transfect suspension 293 or CHO cells in a 30 ml volume. If you wish to transfect the suspension cells in a larger volume, scale up the volume of each reagent in proportion to the culture volume. Refer to Table 1 for details.

1. Cell Seeding

- Approximately 24 hrs before transfection, pass the suspension 293 or CHO cells at ~7x10^5 cells/ml. Place the flask on an orbital shaker platform rotating at 135 rpm at 37° C, 8% CO_2.
- On the day of transfection, the cell density should be about 1.3-1.5 x 10^6/ml. Dilute the cells to 1 x 10^6 /ml.
  Note: To ensure high transfection results, viability of cells must be over 90%.
- Add 30 ml of cells into each 125 ml shake flask.

2. Preparation of LipoD293™-DNA Complex and Transfection Procedures

The optimal ratio of LipoD293™ (µL):DNA (µg) varies from 2:1 to 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.

- Dilute 36 µg of plasmid DNA into serum-free DMEM with High Glucose to a total volume of 0.6 ml and vortex to mix.
- In a separate tube, dilute 108 µl of LipoD293™ Reagent in serum-free DMEM with High Glucose to a total volume of 0.6 ml. Vortex to mix.
- Add diluted LipoD293™ Reagent immediately to the diluted DNA solution to obtain a total volume of 1.2 ml and vortex to mix.
  Important: do not mix the solutions in the reverse order !
- Incubate the DNA-LipoD293™ mixture for 10~15 minutes at room temperature to allow transfection complexes to form.
  Important: Never incubate for longer than 20 minutes
- Add 1.2 ml of transfection complex mixture into the 125 ml cell containing flask with gentle agitation.
- Incubate transfected cell cultures at 37° C, 8% CO_2 on an orbital shaker platform rotating at 135 rpm.
- **Optional:** Remove LipoD293™/DNA complex-containing medium and replace with fresh complete medium 12~18 hours post transfection.
- Check transfection efficiency 24 to 48 hours post transfection.

Table 1. A Scale-up Guideline for Transfecting Suspension 293 or CHO Cells

Culture Volume	Culture Flask	DNA Amount	LipoD293™ Reagent	Diluent Volume
30 ml	125 ml	36 µg	108 µl	2x0.6 ml
250 ml	1000 ml	300 µg	900 µl	2x5.0 ml
1000 ml	3000 ml	1200 µg	3600 µl	2x20 ml

Storage: LipoD293™ DAN In Vitro Transfection Reagent (Ver. II) is stable for up to 12 months at 4 °C. This item shipped at ambient temperature