293Tran™ -
An Efficient DNA Transfection Reagent Specifically for HEK293
Application Guide

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Package Content and Storage Conditions

SKU	Components	Storage Condition	Shipping Condition
TT500001	1 vial of 293Tran, 0.5 mL	+4°C	Room temperature
TT500002	1 vial of 293Tran, 1 mL	+4°C	Room temperature
TT500002P5	5 vials of 293Tran, 1 mL	+4°C	Room temperature

NOTE: FOR RESEARCH PURPOSES ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USAGE.

Related Products

Expression cDNA clones/vectors **-** Tagged and untagged, ready for transfection
shRNA plasmids- Human, Mouse and Rat
CRISPR vectors, gene knockout kits

For technical assistance, contact OriGene at 1-888-267-4436 (301-340-3188 outside the US) or write to us at techsupport@origene.com

Introduction

293Tran is a non-lipid polymer-based transfection reagent specifically formulated for transfecting plasmid DNA efficiently into HEK293 cells.

The major advantages of 293Tran include:

- **High transfection efficiency**
- **Simple application**: Perform better with serum-containing media;

Experimental Procedures

Important Guidelines for Transfection using 293Tran:

1. 293Tran was formulated for DNA transfection ONLY!
2. For high efficiency and lower toxicity, transfect cells at a higher density, 70~80% confluency at the time of transfection.
3. To lower cytotoxicity, transfect cells in the presence of serum (10%) and antibiotics

A General Protocol for Transfecting Adherent HEK293 Cells

A sample protocol is listed here for experiments performed in 24-well plates. If performing experiments in other cell culture plates, simply multiply the suggested quantities by the relative surface area of your plate.

Step I. Cell Seeding:

HEK293 cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal 80% confluency at the time of transfection (around 2x10^5 HEK293 cells).

Note: High serum levels (>5%) with antibiotics usually do not have inhibitory effect on transfection efficiency.

Step II. Transfection

For most cell types, the optimal ratio of 293Tran : DNA is around 3:1 (uL:ug). To ensure the optimal size of complex particles, we recommend using serum-free DMEM to dilute DNA and 293Tran Reagent. **Note: Don't use Opti-MEM!**

The following protocol is for transfection in 24-well plates, refer to **Table 1** for transfection in other culture formats.

1. Change fresh media: 30~60 minutes before transfection, replace cell culture media with 0.5 mL of fresh complete media containing serum and antibiotics.
2. For each well, dilute 0.5 µg of DNA into 50 µl of serum-free DMEM. Gently pipette up and down or vortex briefly to mix.
3. Add 1.5 µl of 293Tran reagent into the diluted DNA (not the reverse order). Gently pipette up and down or vortex briefly to mix. Incubate for 10~15 minutes at room temperature to allow transfection complexes to form.

   Note: Never use Opti-MEM to dilute 293Tran reagent and DNA, it contains serum and will disrupt transfection complex formation.

   Note: Never keep the 293Tran/DNA complexes longer than 20 minutes at room temperature.

4. Add the mixture prepared in step 3 drop-wise onto cells. Gently rock the plate back-and-forth and from side-to-side to distribute the complex evenly. Incubate cells at 37°C.
5. 12~18 hours post transfection, remove transfection complex-containing media and replace with fresh complete serum/antibiotics containing media.
6. Check the transfection efficiency 24 to 48 hours post transfection.

Table 1. Recommended Amounts for Different Culture Plates

Culture Dish	Culture Media (mL)	Plasmid DNA (ug)	Diluent Volume (uL)	293Tran Volume (uL)
48-well	0.3	0.25	50	0.75
12-well	0.75	0.75	76	2.25
6-well	1.0	1	100	3.0
35 mm dish	1.0	1	100	3.0
60 mm dish	2.8	2.5	200	7.5
10 cm dish	5.0	5	500	15

General Protocol for Transfecting Suspension 293 Cells:

Please follow the protocol below to transfect suspension 293 cells. This protocol is for transfecting cells in a 30 mL volume in a shaking flask. If you wish to transfect the suspension cells in a larger volume, adjust the transfection accordingly.

Step I. Prepare cells

1. The day before transfection, determine the numbers of the cells

   Grow cells so that at the day of transfection (roughly 24 hours after) the cell density will reach 10^6/mL cells in 30 mL standard culture media.

2. At the day of transfection, count cell viability and adjust cell density

   The cell density should be 1.0x10^6 per mL in 30 mL standard culture media (total 3x10^7 cells). Place the flask containing cells in a 37°C incubator on an orbital shaker.

Important: For best results, make sure to have a single-cell suspension.
It may be necessary to vortex the cells vigorously for 10–30 seconds to break down cell lumps. The viability of cells must be >90%.

Step II. Transfection

For each transfection, prepare transfection-DNA complexes:

1. Dilute 25 µg of plasmid DNA in serum free DMEM to a total volume of 1 mL. Vortex to mix.

2. Dilute 60 µL of 293Tran™ Reagent in serum free DMEM to a total volume of 1 mL. Vortex to mix.

Note: Never use Opti-MEM to diliute plasmid and 293Tran Reagent because trace of serum from Opti-MEM may interfere with transfection complex formation.

3. Add diluted 293Tran reagent to the diluted DNA to obtain the total volume of 2 mL transfection mix. Vortex to mix.

4. Incubate for 10 minutes at room temperature to allow the formation of DNA-293Tran™ complexes.

   Important: Never leave the DNA-293Tran complex longer than 20 minutes at RT before adding to cells.

5. Add the 2 mL of DNA-293Tran complex to the flask containing 30 mL suspension 293 cells. Incubate cells at 37^{o}C on an orbital shaker rotating at 125 rpm.

6. Check the transfection efficiency 48 hours post transfection.