Product Number: DF37293

DNA-Fect™ *293*

in vitro DNA Transfection Reagent

Instruction Manual

Neuromics
5325 West 74th Street, Suite 8
Edina, MN 55438
Phone: 952-374-6161
Fax: 612-677-3976
Email: pshuster@neuromics.com
Website: www.neuromics.com

Purchaser Notification
Limited License
The purchase price paid for the DNA-Fect™293 Transfection Reagent by end users grants them a non-transferable, non-exclusive license to use the Reagent and/or its separate and included components. This Reagent is intended **for internal research only** by the purchaser. Such use is limited to the transfection of DNA into cells as described in the product manual. Furthermore, research only use means that this Reagent and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of Neuromics.

Purchasers may terminate this License at any time by returning all DNA-Fect293 Transfection Reagent and documentation to Neuromics, or by destroying all Reagent components.

This document covers in full the terms of the DNA-Fect293 Transfection Reagent only license, and does not grant any other express or implied license. The laws of Minnesota shall govern the interpretation and enforcement of the terms of this License.

Product Use Limitations
The Reagent and all of its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the handling of the Reagent components by following appropriate research lab practices.

For more information or any comments on the terms and conditions of this License, please contact:

Pete Shuster
Neuromics
5325 West 74th Street, Suite 8
Edina, MN 55438
Email: **phuster@neuromics.com**

Introduction

in vitro DNA-Fect293 Transfection Reagent contains a patent-pending blend of new gene delivery compounds, which greatly facilitate which greatly facilitate transfection ofDNA to HEK293 cell lines such as HEK293T, HEK293FT, HEK293E, HEK293A.

Important Guidelines

- In order to achieve higher efficiency, transfect cells at high density 90-95% confluency is recommended.
- To minimize cytotoxicity, transfect cells in presence of serum (10%) and antibiotics.
- Change medium with serum (10%) and antibiotics 5 hours post transfection is optional.
- *Storage:* DNA-Fect293 *In Vitro* Transfection Reagent is stable for up to 12 months at +4^{O}C. This product is shipped at ambient temperature.

Procedures for Transfecting Mammalian Cells:

1. For Adherent Cells

Cell Seeding

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches 90-95% confluency at the time of transfection. Freshly complete culture medium with serum and antibiotics is added to each well 30-60 minutes before transfection.

Note: High serum levels (>5%) with antibiotics usually do not have inhibitory effect on transfection efficiency. For some cell lines, higher transfection efficiencies are observed in the presence of serum and antibiotics. We recommend you use complete medium containing serum and antibiotics initially.

Preparation of DNA-Fect293-DNA Complex and Transfection Procedures

For different cell types, the optimal ratio of DNA-Fect293 (µL):DNA (µg) varies from 1:1 to 3:1. We recommend the DNA-Fect293 (µL):DNA (µg) ratio of 3:1 as a starting point which usually gives satisfactory transfection efficiency with little cytotoxicity. To ensure the optimal size of complex particles, we recommend using serum-free DMEM with high glucose to dilute DNA and Transfection Reagent.

The following protocol is 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.

Culture Format	*Transfection Volume (ml)*	*Plasmid DNA (µg)*	*Diluent Volume (µL)*	*DNA-Fect293 (µL)*
96-well	0.2	0.2	2x10	0.6
48-well	0.3	0.5	2x20	1.0
24-well	0.5	1.0	2x50	3.0
12-well	1.0	0.75	2x75	2.5
6-well	1.0	2.0	2x100	6.0
35 mm	1.0	2.0	2x100	6.0
60 mm	2.8	5.0	2x250	15.0
100 mm	6.0	7.0-8.0	2x500	21.0-24.0
T75 flask	8.0	18.0-36.0	2x750	54.0-108.0

Table 1. Recommended Amounts for Different Culture Formats.

24-well Transfection Protocol

- Add 1 µg of DNA into 50 µl of serum-free DMEM with high glucose. Vortex gently and spin briefly to bring drops to bottom of the tube.
- Immediately add the diluted DNA-Fect293 Reagent to the diluted DNA solution. (Important: do not mix the solutions in the reverse order !).
- Vortex the solution immediately. Spin down briefly to bring liquid drops to bottom of the tube.
- Leave the solution undisturbed for 15-20 min. at room temperature to allow DNA-Fect293-DNA complexes to form. Note: *Never keep the DNA-Fect293-DNA complex longer than 20 minutes.*
- Add 100 µl DNA-Fect293-DNA complex drop-wise into each well containing cells and medium. Mix gently by rocking the plate back and forth.
- Change medium 12-18 hours post transfection.

For sensitive cells, to lower cytotoxicity, remove DNA-Fect293-DNA complex and replace with complete medium 5 hours after transfection.

2. For Suspension Cells

The following protocol is given for transfection in 6-well plate. The protocol can be scalded up or down according to culture volume.

Cell Seeding

Suspension cells are typically seeded the day of the transfection at a density of 0.5-1.0 x 10^6 cells per ml of culture. For optimal transfection conditions, seed the number of cells adapted to the culture vessel format according to Table 2.

- 30-60 minutes before transfection, warm fresh medium in a 37^O C water bath. Aspirate out the old medium from each well and add 0.5 ml fresh medium with serum and antibiotics.

Culture Format	*Number of Cells to Seed*
100 mm	5×10^6 -1×10^7
60 mm	2×10^6 -5×10^6
35 mm	5×10^5 -2×10^6
6-well	2×10^5 -5×10^5
24-well	1×10^5 - 2×10^5
48-well	5×10^4 - 1×10^5
96-well	2×10^4 - 5×10^4

Table 2: Recommended Number of Suspension Cells to Seed.

DNA-Fect293-DNA Complex Preparation and Transfection Procedure

For different cell types, the optimal ratio of

Neuromics Antibodies • 5325 West 78th Street, Suite 8 • Edina, MN 55438
phone 952-374-6161 • fax 612-677-3976 • e-mail pshuster@neuromics.com

DNA-Fect293 (µL):DNA (µg) varies from 1:1 to 3:1. We recommend the DNA-Fect293 (µL):DNA (µg) ratio of 3:1 as a starting point which usually gives satisfactory transfection efficiency with little cytotoxicity. To ensure the optimal size of complex particles, we recommend using serum-free DMEM with high glucose to dilute DNA and Transfection Reagent.

6-well Transfection Protocol

- Add 2 µg of DNA into 100 µl of serum-free DMEM with high glucose. Vortex gently and spin briefly to bring drops to bottom of the tube.
- For each well, dilute 6 µl of DNA-Fect293 Reagent into 100 µl of DMEM Serum-free Medium with High Glucose. Vortex gently and spin down briefly.
- Add 100 µl of DNA-Fect293 immediately to the 100 µl DNA solution all at once (Important: do not mix the solutions in the reverse order !).
- Vortex the solution immediately. Spin down briefly to bring liquid drops to bottom of the tube.
- Incubate 15-20 minutes at room temperature.
- Add 200 µl DNA-Fect293-DNA complex drop-wise into each well containing cells and medium. Mix gently by rocking the plate back and forth.
- Incubate at 37^{O}C and 5% CO2 in a humidified atmosphere.
- Transfection experiments are usually stopped after 24 to 48 hours and gene activity assessed. Cells growing in suspension are collected by centrifugation at 800 x g and then re-suspended in the desired medium or buffer.

Neuromics Antibodies • 5325 West 78th Street, Suite 8 • Edina, MN 55438
phone 952-374-6161 • fax 612-677-3976 • e-mail pshuster@neuromics.com