Product Number: DF37100

DNA-Fect™

in vitro DNA Transfection Reagent

Instruction Manual

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Introduction

in vitro DNA-Fect Transfection Reagent contains a patent-pending blend of new gene delivery compounds, which greatly facilitate transfection of DNA to various established cell lines as well as primary cells.

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-Fect *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-Fect-DNA Complex and Transfection Procedures

For different cell types, the optimal ratio of DNA-Fect (µL):DNA (µg) varies from 1:1 to 3:1. We recommend the DNA-Fect (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-Fect (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-Fect 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-Fect-DNA complexes to form. Note: *Never keep the DNA-Fect-DNA complex longer than 20 minutes.*
- Add 100 µl DNA-Fect-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-Fect-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 x 10^6 -1 x 10^7
60 mm	2 x 10^6 -5 x 10^6
35 mm	5 x 10^5 -2 x 10^6
6-well	2 x 10^5 -5 x 10^5
24-well	1 x 10^5 - 2 x 10^5
48-well	5 x 10^4 - 1 x 10^5
96-well	2 x 10^4 - 5 x 10^4

Table 2: Recommended Number of Suspension Cells to Seed.

DNA-Fect-DNA Complex Preparation and Transfection Procedure

For different cell types, the optimal ratio of DNA-Fect (µL):DNA (µg) varies from 1:1 to 3:1. We recommend the DNA-Fect (µL):DNA

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(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-Fect Reagent into 100 µl of DMEM Serum-free Medium with High Glucose. Vortex gently and spin down briefly.
- Add 100 µl of DNA-Fect 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-Fect-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.

Transfecting Hard-to-Transfect Mammalian Cells:

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.

1. Cell Culture

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.

Culture Format	*Surface Area (cm^2)*	*Number of Cells to Seed*
T75 Flask	75	9.6 x 10^6
100 mm	58	7.3 x 10^6
60 mm	21	2.7 x 10^6
35 mm	9.6	1.2 x 10^6
6-well	9.6	1.2 x 10^6
12-well	3.5	0.44 x 10^6
24-well	1.9	0.24 x 10^6
48-well	1.0	0.11 x 10^6
96-well	0.3	0.35 x 10^5

Table 3. A Guideline for Optimal Cell Number Per Well in Different Culture Formats.

Culture Format	*Transfection Volume (ml)*	*Plasmid DNA (µg)*	*DNA-Fect (µL)*
96-well	0.02	0.2	0.8
48-well	0.04	0.5	2.0
24-well	0.1	1.0	4.0
12-well	0.2	2.0	8.0
6-well	0.2	2.0	8.0
60 mm	0.5	5.0	20.0
100 mm	2.8	8.0	32.0
T75 flask	1.0	36.0	144.0
250 ml flask	2.5	100.0	400.0

Table 4. Recommended Amounts for Different Culture Vessel Formats.

2. Preparation of Cells in Suspension

The following protocol is given for transfecting hard-to-transfect mammalian cells in 6-well plates, refer to Table 3 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 1.2x10^6 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.

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3. Preparation and Application of Transfection Complex.

For hard-to-transfect mammalian cells, the optimal ratio of DNA-Fect (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 DNA-Fect Reagent.

6-well Transfection Protocol

- 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 DNA-Fect reagent into 100 µl of serum-free DMEM with high glucose. Vortex gently and spin down briefly.
- Add the diluted DNA-Fect 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 2 immediately in the 200 µl transfection complex and incubate at 37 °C for 20 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 5-12 hours after plating.
- Check transfection efficiency 24 to 48 hours post transfection.

Protocol for generation of Lentivirus from 293T cells

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.

Cell Seeding

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal ~95% 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: High serum levels (>5%) with antibiotics usually do not have inhibitory effect on transfection efficiency. For some specific 293 cells, maximal transfection efficiencies are observed in the presence of serum and antibiotics. We recommend using complete serum/antibiotics-containing medium initially.*

Culture Format	*Surface Area (cm^2)*	*Number of Cells to Seed*
T75 Flask	75	3.0-6.0 x 10^6
100 mm	58	2.2-4.4 x 10^6
60 mm	21	0.9-1.8 x 10^6
35 mm	9.6	3.5-7.0 x 10^5
6-well	9.6	4.0-8.0 x 10^5
12-well	3.5	1.5-3.0 x 10^5
24-well	1.9	0.8-1.6 x 10^5
48-well	1.0	4.0-8.0 x 10^4
96-well	0.3	1.2-2.4 x 10^4

Table 5. A Guideline for Optimal Cell Number Per Well in Different Culture Formats.

Preparation of DNA-Fect-DNA Complex and Transfection Procedures

The following protocol is given for transfection in 10 cm dish. For other culture formats, scale up or down per culture dish's surface. The optimal transfection conditions are given in the standard protocol described below.

- Cell confluency should be ~95 % at the day of transfection
- For each 10 cm dish, add 5.0 ml of complete medium with serum and antibiotics freshly 30~60 minutes before transfection.
- For each dish, dilute total 45 µg of DNA (three DNAs with 15 µg each) into 500 µl of serum-free DMEM with high glucose. Vortex gently and spin down briefly to bring drops to bottom of the tube.
- For each dish, dilute 60 µl of DNA-Fect Reagent into 500 µl of serum-free DMEM with high glucose.
- Vortex gently and spin down briefly.
- Add the diluted DNA-Fect Reagent immediately to the diluted DNA solution all at once. (Important: do not mix the solutions in the reverse order !)

- Vortex- mix the solution immediately and spin down briefly to bring drops to bottom

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of the tube followed by incubation of 15~20 minutes at room temperature to allow DNA-Fect complexes to form. *Note: Never keep the DNA/DNA-FECT complex longer than 20 minutes*

- Add the 1000 µl DNA-Fect / DNA complex drop wise onto the medium in each dish and homogenize the mixture by gently swirling the plate.
- Remove DNA/DNA-Fect complex-containing medium and replace with fresh complete serum/antibiotics containing medium 5 hours post transfection.
- Check transfection efficiency and virus titer 24 to 48 hours post transfection. 48 hours gives better titers.

Procedures for Transfecting Bacmids into Sf9 Cells

1. Count Sf9 cells, and adjust cell density to 5 x10^5 cells/ml in unsupplemented SF900II media
2. Seed 2 ml of cell suspension per well (1 x 106 cells/well).
3. Label 2 wells as "negative control", 2 wells as "1 µg DNA", and 2 wells as "2 µg DNA"
4. Incubate dishes at 27°C for 30-60 minutes (enough time to allow the cells to attach to the bottom of the wells).
5. Aliquot 500 µl of sterile diluent (150 mM NaCl) into three 1.5 ml Eppendorf tubes. Label the tubes "0", "1 µg", and "2 µg". These will serve as 2.5X Master Mixes for each of the three conditions. *NOTE: The sterile diluent should be 150 mM NaCl which is essential for DNA/DNA-Fect complex formation. IT IS IMPORTANT THAT THE DNA IS ADDED FIRST AND THE DNA-Fect Reagent IS ADDED SECOND TO EACH TUBE.*
6. Aliquot 2.5 µg of bacmid into the "1 µg" Master Mix tube.
7. Aliquot 5 µg of bacmid into the "2 µg" Master Mix tube.
8. Briefly vortex the tubes.
9. To the "1 µg" Master Mix tube, add 10 µl of DNA-Fect and IMMEDIATELY VORTEX for 5 seconds.
10. To the "2 µg" Master Mix tube, add 20 µl of DNA-Fect Reagent and IMMEDIATELY VORTEX for 5 seconds.
11. Allow the Master Mix tubes to sit in the hood for 10-15 minutes.

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