ExFect2000 Transfection Reagent

Catalog # T202

Version 7.1

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1. Introduction

The ExFect2000 Transfection Reagent is a novel and efficient transfection reagent based on cationic liposomes, which is suitable for DNA transfection and co-transfection systems for most eukaryotic cells (both adherent and suspended). The ExFect2000 has a unique structure and an optimized formulation. The presence of serum and antibiotics will not affect the efficiency of transfection, thereby reducing the effect of serum-deprival on cells. The ExFect2000 is also with low cytotoxicity. After transfection, there's no need to remove nucleic acid-ExFect2000 complex or replace medium within 24 hr-48 hr. The high transfection efficiency of ExFect2000 transfection reagent has been validated in a board range of eukaryotic cell lines.

2. Package Information

Components	T202–01	T202–02	T202–03
ExFect2000 Transfection Reagent	0.5 ml	1 ml	5 ml

3. Storage

Stored at 2-8℃. **DO NOT Freeze!**

Note: Gently turn the tube upside down to mix thoroughly before use. Vortex if there are insoluble substances in the reagent. If they are still not dissolved, please use after centrifugation at 1000 rpm.

4. Application

The ExFect2000 has been validated in the following cell lines: HEK 293, 293T, A549, B16F10, HCT116, WRL68, MDA-MB-231, Vero, HeLa, Hepa 1-6, Hepa 1cLc7, HepG2, BHK-21, BNLCL.2, C2C12, C6, CHO K1, COS-1, COS-7, Daoy, DU 145, K562, KB, LL/2 (LLC1), LNCaP-FGC, MCF-7, MEL, Neuro 2a, NIH3T3, OVCAR-3, PC-3, and PC-12.

This product is for research use only, not for use in clinical diagnosis and treatment.

5. Additional Materials Required

Plasmid DNA extraction: QIAGEN Plasmid Maxi Kit or other equivalent products;

Cell culture: Cell lines (from ATCC or other reliable sources), Cell culture medium (i.e. Gibco), FBS, Trypsin, Cell Counting Chamber, Cell culture plates, etc.

ExFect2000/DNA packaging: It is recommended to use opti-MEM (or other serum-free and antibiotic-free cell culture medium), 1.5 ml EP tube, etc.

Other materials: PBS, Pipette, 15 ml centrifuge tube, T-75 cell culture flask, Carbon dioxide cell incubator, etc.

6. Notes

6-1. The transfection efficiency depends on cell density. To improve reproducibility, please use a constant number of cells for each transfection.

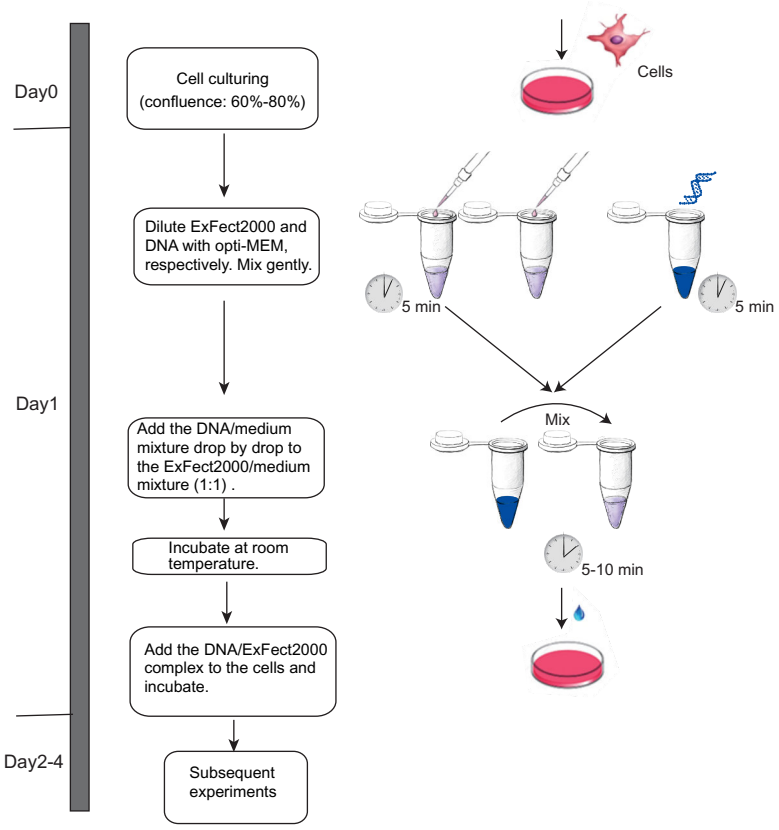
6-2. The mixing of ExFect2000 with opti-MEM and the mixing of DNA with opti-MEM should be performed separately, followed by gentle mixing of the ExFect2000/medium mixture with the DNA/medium mixture. Avoid the presence of serum during this packaging reaction, as serum interferes with the formation of ExFect2000/DNA complex.

6-3. During packaging, add the DNA/medium mixture drop by drop to the ExFect2000/medium mixture.

6-4. Gently handle the solutions during transfection.

6-5. Use high quality DNA (with high purity, sterile, and endotoxin-free) for transfection.

7. Transfection Workflow



8. Protocol

08-1. Key Factors for Transfection Efficiency

(1). Cell medium: ExFect2000 is compatible with most cell culture medium. there is no need to replace the medium before or after the transfection. Serum does not impact the transfection.

(2). Cell density: Generally, the highest transfection efficiency can be obtained when cell density is 60%-80%. However, the optimal cell density, which varies between different cell lines, can be determined via pre-experiments.

(3). DNA purity and concentration: DNA for transfection should be of high purity and contain no endotoxin. It is recommended to add 0.5 μg-1 μg of DNA per well for a 24-well plate transfection. For other culture volumes, please refers to **Table 1** (Section 08-2).

(4). Ratio of DNA/ExFect2000: It is recommended to start with an initial ratio of 1:2 or 1:3 (i.e. mix 1 μg of DNA with 2 μl or 3 μl of ExFect2000). Adjust this ratio from 1:1 to 1:3 to obtain the optimized transfection efficiency.

(5). Incubation time after transfection: Incubation of 24 to 48 hours is enough for most cell lines. In special cases, the incubation time can be optimized within 12 to 72 hours.

08-2. Optimizing Transfection System

The volume of medium and the amount of DNA or ExFect2000 can be optimized according to **Table 1**.

Table 1. Initial transfection conditions recommended for different culture systems.

		96-well Plate	48-well Plate	24-well Plate	12-well Plate	6-well Plate	10 cm Dish
Surface Area (cm^2)*		0.35	1	1.9	3.8	9.6	59
Complex Formation Reaction	Serum-free Media (μl)	10	25	50	100	250	2000
	ExFect2000 (μl)	0.4	1	2	4	10	60
	1 μg/μl plasmid (μl)	0.2	0.5	1	2	5	30
Complete Growth Media (ml)		0.10	0.25	0.5	1.0	2.0	10

*Data from Greiner tissue culture and Falcon 10 cm dishes.

08-3. Transient Transfection of Adherent/Suspension cells (in 24-well plates)

◇ **Cell Culture**

One day before transfection, plate appropriate number of cells so that cells will be 60% -80% confluent at the time of transfection.

◇ **Prepare the DNA/ExFect2000 Complex**

(1). Add 25 µl of serum-free medium into a 1.5 ml sterile centrifuge tube. Then add ExFect2000 transfection reagent with an appropriate volume (1 µl-3 µl ExFect2000/µg DNA, refer to **Table 1**). Mix gently by pipetting and incubate at room temperature for 5 min.

(2). Add 25 µl of serum-free medium into a 1.5 ml sterile centrifuge tube. Then, add DNA with an appropriate volume (0.5 µg-1 µg DNA/well, refer to **Table 1**). Mix gently by pipetting and incubate at room temperature for 5 min.

(3). Add the DNA/medium mixture drop by drop to the ExFect2000/medium mixture. Mix gently by pipetting and incubate at room temperature for 5-10 min. Start transfection immediately.

The DNA /ExFect2000 complex should be used within 60 min.

Do not reverse the mixing order of the ExFect2000/medium mixture and the DNA/medium mixture.

◇ **Transfection**

(1). Add the DNA/ExFect2000 complex drop by drop to each well containing cell and medium. Mix gently rocking the plate back and forth.

If necessary, replace the culture medium before transfection.

(2). Incubate cells in a CO_2 incubator for 24 to 48 hours.

(3). Harvest cells for subsequent experiments.

9. Trouble Shooting

Low transfection efficiency

(1) The ration of DNA/ExFect2000 or the amount of DNA is not optimized.

Optimize the ratio of DNA/ExFect2000 (in a range of 1 µl-3 µl ExFect2000/µg DNA) and the optimal DNA amount (in a range of 0.5 µg-1 µg/well for a 24-well plate) by pre-experimentation. For more DNA amounts, refer to Table 1. Select a ratio with high transfection efficiency and low cytotoxicity for transfection.

(2) The ExFect2000 and DNA are mixed directly with serum-free medium.

Dilute ExFect2000 and DNA respectively in serum-free medium, and then incubate together.

(3) The cell density is not optimal.

Generally, the highest transfection efficiency can be obtained when cell density is 60%-80%. However, the optimal cell density, which varies between different cell lines, can be determined via pre-experiments.

(4) Low quality DNA (degrade or containing endotoxin).

DNA used for transfection should be of high purity, sterile, and of no endotoxin.

(5) The DNA/ExFect2000 complex packaging system contains serum.

Serum interferes with the formation of the DNA/ExFect2000 complex.

(6) Transfection inhibitory factors exist in the transfection system.

Transfection will not proceed normally with polyanionic polymers (i.e. dextran sulfate, heparin) in the transfection system. It is recommended to use media without these components.

(7) Poor cell status

Select moderately passaged cell lines and maintain the same passage times in different experiments for high efficiency and low cytotoxicity.

Poor cell status after transfection

(1) Long incubation time after transfection with DNA/ExFect2000 complex

In most cases, there's no need to replace the medium in 24 hours after transfeciton. However, prolonged incubation may lead to poor cell status. It is recommended to arrange the follow-up experiment time according to demands.

(2) Cell density is not optimal

Generally, the highest transfection efficiency can be obtained when cell density is 60%-80%. Low cell density leads to slow cell growth and higher sensitivity on foreign stimuli. While high cell density leads to contact inhibition and accelerated apoptosis.

(3) The ratio of DNA/ExFect2000 or the amount of DNA is not optimized.

Excessive transfection reagent results in higher cytotoxicity. Try to reduce the use of transfection reagents or reduce the amount of DNA (refer to **Table 1**).

(4) Unevenly distribution of DNA/ExFect2000 complex in culture medium

Excessive regional distribution of DNA/ExFect2000 complex is one of the most common causes of high cytotoxicity. After adding the complex to culture medium, mix gently rocking the plate back and forth for several times.

(5) Poor cell status

Select moderately passaged cell lines and maintain the same passage times in different experiments for high efficiency and low cytotoxicity.