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Chemi - Trans™ Lipofectin 2000 Transfection Reagent

(Cat#: T002, T003)

Introduction:

The Chemi - Trans™ Lipofectin 2000 Transfection Reagent is a proprietary lipid-based transfection reagent that forms a complex with DNA or small RNA, and can efficiently deliver the complex into a broad range of commonly used mammalian cell lines. Chemi - Trans™ Lipofectin 2000 Transfection Reagent has been proven to get a good performance as well as Lipofectamine® 2000 Reagent in DNA and siRNA transfection.

Advantages:

- ❖ Good transfection efficiency and low cytotoxicity.
- ❖ Provides a convenient transfection for both plasmid DNA and small RNA.
- ❖ Compatible with serum and antibiotics in culture medium.

Table 1: Product Package & Storage

Cat #	Product Name	Volume	Storage
T002	Chemi -Trans™ Lipofectin 2000 Transfection Reagent	1.0 mL	2 ~ 8 °C, stable for up to 12 months when stored appropriately. (DO NOT FREEZE.)
T003		3.0 mL	

Important Guidelines for Transfection:

(1) For maximum transfection efficiency, using serum-free medium (such as Opti-MEM® Reduced Serum Medium) to dilute DNA is a must.

(2) While the standard protocols for DNA transfection being given below, optimization is often needed for maximal transfection efficiency.

Standard Protocol for DNA Transfection of Adherent Cells

Step Ⅰ. Cell Seeding:

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to 70 ~ 90% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30 ~ 60 min before transfection.

Step Ⅱ. DNA Transfection Protocol

Use the following procedure to transfect DNA into mammalian cells in a 6-well format. For other formats, please refer to ***A Guideline for DNA transfection (Table 2)***. All amounts and volumes are given on a per well basis. For each transfection sample, prepare complexes as follows:

a. Dilute 2.5 μg Endotoxin-free plasmid DNA in 125 μL of serum-free Opti-MEM (or other medium without serum), Vortex to mix gently but thoroughly.

b. Mix Chemi - Trans™ Lipofectin 2000 Reagent gently before use, then dilute 3.75 μL ~ **10.0 μL (for difficult-to-transfect cells)** of Chemi - Trans™ Lipofectin 2000 in 125 μL of serum-free Opti-MEM.

c. Add the diluted DNA mixture (prepared in Step a.) to the diluted Chemi - Trans™ Lipofectin 2000 Reagent all **(1:1 ratio)** after the diluted formulation be incubated for 5 minutes, and mix by pipetting up and down. Incubate for 10 ~ 15 minutes at room temperature to let transfection complex form well.

d. Add the 250 μL of complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth.

e. Incubate cells at 37°C in a CO2 incubator for 24 to 72 hours. Then, to measure the gene silencing by qRT-PCR and Western Blotting respectively.

Table 2: A Guideline for DNA transfection per cell culture vessel

Culture Vessel	Growth Medium (mL)	Serum - Free Medium (μL)	DNA (μg)	Chemi - Trans™ Lipofectin 2000 Reagent (μL)
	Volume used per well			
96-well	0.1	2 × 5	0.1	0.15 ~ 0.4
24-well	0.5	2 × 25	0.5	0.75 ~ 2.0
12-well	1.0	2 × 50	1.0	1.5 ~ 4.0
6-well	2.0	2 × 125	2.5	3.75 ~ 10.0

**[1] We strongly suggest that keep the concentration of plasmid DNA be 0.5 ~ 2.0 μg/μL, and the Endotoxin-free plasmid is extremely important for a successful transfection.*

[2] Transfect plasmid DNA and small RNA at the same time using Chemi - Trans™ Lipofectin 2000 Reagent by adding 30 pmol small RNA per 1 μg DNA. It is kindly suggested to stock the small RNA solution at 10 μM.

[3] To obtain a robust transfection efficiency and low cytotoxicity, optimize transfection conditions by varying cell density as well as DNA and Chemi - Trans™ Lipofectin 2000 Reagent concentrations. Ensure that cells are greater than 90% confluent and vary DNA (μg): Chemi - Trans™ Lipofectin 2000 Reagent (μL) ratios from 1:1 to 1:4.

[4] You may perform a rapid 96-well plate transfections by plating cells directly into the transfection complexes. Prepare complexes in the plate and directly add cells drop wise at twice the cell density as in the basic protocol in the complete growth medium. Cells will adhere as usual in the presence of complexes.