Lipofectin 3000 Transfection Reagent

Cat No.:KF-LP030

Introduction:

The Lipofectin3000 Transfection Reagent is a lipid-based transfection reagent that forms a complex with DNA or small RNA, and can efficiently deliver the complex into a variety of adherent and suspension mammalian cell lines. Lipofectin3000 Transfection Reagent has been tested to get a good performance as well as Lipofectamine® 3000 Reagent in DNA and siRNA transfection,

Advantages:

❖ Robust transfection efficiency and low cytotoxicity.

❖ Excellent for difficult-to-transfection cells, such as some primary cells, macrophage cells, suspension cell lines and stem cells.

❖ Compatible with serum and antibiotics in culture medium.

Storage：

2 ~ 8 °C, stable for up to 12 months

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.

StepⅡ. 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), and then add 5.0 μL Enhancer Reagent. Vortex to mix gently but thoroughly.

b. Mix Lipofectin Reagent gently before use, then dilute 3.75 μL ~ 7.5 μL (for difficult-to-transfect cells) of Lipofectin in 125 μL of serum-free Opti-MEM.

c. Add the diluted DNA mixture (prepared in Step a.) to the diluted 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 CO_2 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)	Component B Enhancer Reagent (μL)	Component A Reagent (μL)
	Volume used per well				
96-well	0.1	2 × 5	0.1	0.2	0.15~0.3
24-well	0.5	2 × 25	0.5	1.0	0.75~1.5
12-well	1.0	2 × 50	1.0	2.0	1.5~3.0
6-well	2.0	2 × 125	2.5	5.0	3.75~7.5

[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. Do not add Enhancer Reagent when dilute the siRNA solution (Step a) for siRNA transfection with Lipofectin Reagent.

[2] To obtain a robust transfection efficiency and low cytotoxicity, optimize transfection conditions by varying cell density as well as DNA and Lipofectin Reagent concentrations. Ensure that cells are greater than 90% confluent and vary DNA (μg): Enhancer Reagent (μL): Lipofectin Reagent (μl) ratios from 1:2:1 to 1:2:4

[3] 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.

Important Guidelines for Transfection:

(1) For maximum transfection efficiency, using serum-free medium (such as Opti-MEM® Reduced Serum Medium) to dilute DNA and the Lipofectin3000 Transfection Reagent is a must.

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

(3) It is unnecessary to wash cells and change medium after transfection.

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