DNA Transfection Reagent

Cat No.:KF-LP010

Introduction:

The DNA Transfection Reagent is formulated as a biodegradable polymer-based DNA delivery tool that performs well for DNA transfecting in a variety of commonly used and hard-to-transfect mammalian cell lines. A remarkable feature of Reagent is the rapid and complete degradation of polymer after transfection complex by endocytosis, leading to much less cytotoxicity.

Advantages:

- Bio-degradable and low cytotoxicity.
- Excellent for long DNA transfection.
- Easy to use, forms a DNA complex in just five minutes.
- 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 ~ 80% 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 2.0 μg Endotoxin-free plasmid DNA in 100 μL of serum-free DMEM with High Glucose. Vortex to mix gently but thoroughly.

b. Mix Reagent gently before use, then dilute 6.0μL of Reagent in 100 μL of serum-free DMEM with High Glucose.

c. Add the diluted Reagent immediately to the diluted DNA mixture (prepared in Step a, do not mix the solutions in reverse.) 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 200 μL of complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth. Incubate cells at 37°C in a CO2 incubator.

e. Remove the Reagent/DNA complex-containing medium and replace with pre-warmed fresh complete serum/antibiotics containing medium 4 ~ 6 hours post transfection when it is necessary. Then, to measure the gene silencing by qRT-PCR and Western Blotting respectively24 ~ 72 hours post transfection.

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

Culture Vessel	Growth Medium (mL)	Serum - Free Medium (μL)	DNA (μg)	DNA transfection 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 × 100	2.0	3.0~8.0

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

[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 serum-free DMEM with High Glucose. DO NOT use Opti-MEM Medium, it contains serum and will disrupt transfection complex) to dilute Reagent and DNA is a must.

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

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