

LiFect3K™ Transfection Reagent

Cat. #: M0004 Size: 0.75 ml/1.5 ml

Introduction

LiFect3K™ Transfection Reagent is a lipid-based transfection reagent that forms a complex with DNA or RNA, and transports the complex into a variety of adherent and suspension cell lines. This reagent delivers superior transfection efficiency and improved cell viability for the widest range of hard-to-transfect and common cells. LiFect3K™ Reagent has been tested to work the same efficiency as **Lipofectamine® 3000 Reagent**, and used for the transfection of both DNA and RNA into eukaryotic cells even in the presence of serum.

Package Information

Components	M0004-01	M0004-02
LiFect3K™ Reagents	0.75 ml	1.5 ml
Enhancer Reagent	0.75 ml	1.5 ml

Storage

Store at 4°C

Protocols

Use the following procedure to transfect DNA into mammalian cells in a 24-well format. For other formats, see Scaling Up or Down Transfections. All amounts and volumes are given on a per well basis. Transfect cells at high cell density for high efficiency, high expression levels, and to minimize cytotoxicity. Optimization may be necessary (see Optimizing Transfection).

1. Adherent cells: One day before transfection, plate 0.5×10^5 cells in 500 µl of growth medium without antibiotics so that cells will be 70-90% confluent at the time of transfection.

Suspension cells: Just prior to preparing complexes, plate 4×10^5 cells in 500 µl of growth medium without antibiotics.

2. For each transfection sample, prepare complexes as follows:

a. Mix LiFect3K™ Reagent gently before use, then dilute 1 µl of LiFect3K™ Reagent in 25 µl of Opti-MEM® I Medium. Incubate at room temperature.

- Dilute 0.5 µg DNA in 25 µl of Opti-MEM® I Reduced Serum Medium without serum (or other medium without serum). Then add 1 µl of Enhancer Reagent. Mix gently and incubate at room temperature.
- Add diluted DNA/Enhancer Reagent mixture to the diluted LiFect3K Reagent (total volume=50 µl). Mix gently and incubate for 10-15 minutes at room temperature.
- Add the 50 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 for 2-4 days. Then, analyze transfected cells. Medium may be changed after 4-6 hours.

Optimizing Transfection

To obtain the highest transfection efficiency and low cytotoxicity, optimize transfection conditions by varying cell density as well as DNA and LiFect3K™ Reagent concentrations. Make sure that cells are greater than 90% confluent and vary DNA (µg): LiFect3K™ Reagent (µl): Enhancer Reagent (µl) ratios from 1:1:2 to 1:4:2.

Scaling Up or Down Transfections

To transfect cells in different tissue culture formats, vary the amounts of LiFect3K™ Reagent, Enhancer Reagent, nucleic acid, cells, and medium used in proportion to the relative surface area, as shown in the table.

Table 1. A Guideline for Optimal DNA Per Well in Different Culture Formats

Culture Dishes	Surface Area (cm ²)	Plating medium volume	Dilution medium volume	DNA	LiFect3K™	Enhancer Reagent
96-well	0.3	100 µl	2× 5 µl	0.1 µg	0.15~0.3 µl	0.2 µl
24-well	2	500 µl	2× 25 µl	0.5 µg	0.75~1.5 µl	1 µl
12-well	4	1 ml	2× 50 µl	1.0 µg	1.5~3.0 µl	2 µl
6-well	10	2 ml	2× 125 µl	2.5 µg	3.75~7.5 µl	5 µl