Lipofect
Transfection Reagent

For transfection of eukaryotic cells
with nucleic acids

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Lipofect Transfection Reagent

Cat. No. RC01

Kit Contents

Cat.no.	Size
RC0101	0.5 ml
RC0102	5 ml

Storage

Lipofect Transfection Reagent should be stored at 4°C, and is stable for at least 12 months. Lipofect Transfection Reagent should not be frozen.

Introduction

Lipofect Transfection Reagent is a cationic lipid formulation for transfecting nucleic acids into eukaryotic cells.
It has high transfection efficiency in many cell types, especially for cell lines (COS-7, CHO and 293) for protein expression. This reagent could be added directly to cells in culture medium. It is not necessary to remove complexes or change/add medium after transfection.

Important notes before starting

1. When preparing the complexes, the proportion of DNA (μg) and Lipofect (μl) could be 1:0.5 to 1:5. We recommend 1:2 to 1:3 for most of the cells.
2. Best transfection results are obtained using confluence levels of 70-90% for adherent cells or densities of 2×10^6- 4×10^6/ml for suspension cells.
3. Do not add antibiotics to media during transfection since this causes cell death.
4. Test serum-free media for compatibility with Lipofect since some serum-free formulation (e.g. CD293, 293SFM II, VP-SFM) may

inhibit cationic lipid-mediated transfection.

Protocol

Use the following procedure to transfect mammalian cells incubated in a 24-well. For other formats, see **Scaling Up or Down Transfections**.

1. Adherent cell: on the day before transfection, plate 0.5-2×10^5 cells in 500 µl of growth medium without antibiotics so that they will be 90–95% confluent at the time of transfection.
   Suspension cells**:** before the preparing of complexes, plate 4-8 × 10^5 cells in 500 µl of growth medium without antibiotics**.**
2. For each well of cells, dilute 0.8-1.0 µg DNA into 50 µl serum-free medium.
3. For each well of cells, dilute 1-3 µl Lipofect Transfection Reagent into 50 µl serum-free medium; incubate for 5 min at room temperature.
   Note: Mix with diluted DNA within 30 min after Lipofect dilution, excessive incubation may result in decreased activity.
   Note: Mix with diluted DNA within 5 min if the Lipofect is diluted by D-MEM.
4. Combine the diluted DNA with diluted Lipofect (total volume = 100 µl). Mix gently and incubate for 20 minutes at room temperature (solution may appear cloudy).
   Note: Complexes are stable for 6 hours at room temperature.
5. Add the 100 µl of complexes to a well containing cells and medium. Mix gently by rocking the plate back and forth.
   Note: If transfected under serum-free condition, please seed with the medium containing serum. Before adding complexes, replace the medium with 0.5 ml serum-free medium.
6. Incubate cells at 37°C in a CO_2 incubator for 24–48 hours prior to testing for transgene expression. It is not necessary to change the

medium, but medium may be replaced after 4 hours.

7. 24-72 hours after the complex adding, analyze the cell extract or *in situ* cell stain to detect the activity of report gene. To the stable expression cell lines, dilute the plated cell with proportion of 1:10 with fresh medium after the first day of transfection, and add antibiotics on the following day. It will take few days or weeks for the stable expression.

   Suspension cells: 4 hours after the complex adding, add PMA/PHA (if that is necessary)

   Jurkat cells: Add PHA-L and PMA separately to a final concentration of 1 μg/ml and 50 ng/ml to enhance the activity of CMV promoter and gene expression.

   K562 cells: Add PMA to enhance the promoter activity.

Scaling Up or Down Transfections

To transfect cells in different tissue culture formats, vary the amounts of Lipofect transfection Reagent, DNA, cells, and medium used in proportion to the relative surface area, as shown in the following table.

Table. Reaction setting up reference

Culture vessel	Surf. area per well (cm^2)	Relative surf. area vs. 24-well	Vol. of plating medium	DNA (μg) in media vol. (μL)	Lipofect (μL) in media vol. (μL)
96-well	0.3	0.2	100 μl	0.2 μg in 25 μl	0.5 μl in 25 μl
24-well	2	1	500 μl	0.8 μg in 50 μl	2.0 μl in 50 μl
12-well	4	2	1 ml	1.6 μg in 100 μl	4.0 μl in 100 μl
35 mm	10	5	2 ml	4.0 μg in 250 μl	10 μl in 250 μl
6-well	10	5	2 ml	4.0 μg in 250 μl	10 μl in 250 μl
60 mm	20	10	5 ml	8.0 μg in 0.5 ml	20 μl in 0.5 ml
10 cm	60	30	15 ml	24 μg in 1.5 ml	60 μl in 1.5 ml