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by life technologies

Lipofectamine® LTX and PLUS™ Reagents

Catalog Numbers:	Lipofectamine® LTX Reagent	PLUS™ Reagent
A12621	100 µL	40 µL
15338-100	1 mL	0.85 mL

Store both reagents at 4°C (do not freeze)

Description

Lipofectamine® LTX Reagent is a proprietary, animal-origin free cationic lipid formulation for the transfection of DNA into eukaryotic cells that offers the highest transfection expression performance with **low cytotoxicity** to many cell types and formats (e.g., 96-well). Reduced cytotoxicity allows the use of a greater range of lipid doses, and enables excellent transfection results despite differences in cell density, minor pipetting inaccuracies, and other variations.

For many cell lines, addition of the PLUS™ Reagent (also available separately, Cat. no. 11514-015) enhances transfection performance.

Important Guidelines for Transfection

- The addition of antibiotics to media during transfection may result in cell death in some cell lines. Test each cell line individually.
- Lipofectamine® LTX Reagent performs well with vector-based RNAi experiments. For siRNA and Stealth™ RNAi transfections, we recommend Lipofectamine® RNAiMAX Reagent (Cat. no. 13778-075).
- Visit **www.invitrogen.com/transfection** or contact Technical Support for specialized transfection protocols (including cell-type specific advice on use of PLUS™ Reagent and antibiotics, and a protocol for vector-based RNAi).
- We recommend Opti-MEM® I Reduced Serum Medium (Cat. no. 31985-062) for diluting DNA and Lipofectamine® LTX Reagent before complexing.
- Maintain the same seeding conditions between experiments. Transfection can be performed both in the presence or absence of serum. Test serum-free media for compatibility with Lipofectamine® LTX Reagent.

Intended Use

For research use only. Not intended for human or animal diagnostic or therapeutic uses.

Part no. 100009379 MAN0002694 Rev. Date: 11 Apr 2011

Optimization Protocol

Before starting

Prepare a full 24-well plate with the cells to be transfected. Grow adherent cells in 500 µL of growth medium. They should be 50–80% confluent at the time of transfection. Suspension cells should be at a density of 100,000–250,000 cells in 500 µL of growth medium.

Dilute DNA and PLUS™ Reagent

1. Add 20 µg of plasmid DNA to 2 mL of Opti-MEM® I Reduced Serum Medium in a 15-mL conical tube to prepare a 10 µg/mL stock of DNA.
2. Add 20 µL of PLUS™ Reagent to the 15-mL conical tube containing the diluted DNA.
3. Mix well by pipetting or vortexing and incubate the tube at room temperature for 10 minutes.

Dilute Lipofectamine® LTX Reagent

1. Add 300 µL of Opti-MEM® I Reduced Serum Medium into each well of row A of a 24-well plate (complexation plate).
2. Add 3–18 µL of Lipofectamine® LTX Reagent to wells A1–A6 of the complexation plate as outlined in the diagram below.

	1	2	3	4	5	6
A	3 µL	6 µL	9 µL	12 µL	15 µL	18 µL
B	—	—	—	—	—	—
C	—	—	—	—	—	—
D	—	—	—	—	—	—

3. Mix each well by pipetting the solution up and down.

Prepare transfection complexes

1. Add 300 µL of DNA:PLUS™ Reagent solution to wells A1–A6 of the 24-well complexation plate containing diluted Lipofectamine® LTX in Opti-MEM® I Reduced Serum Medium.
2. Mix the contents of each well gently by pipetting up and down 3 times.
3. Incubate the complexation plate at room temperature for 30 minutes.

Add transfection complexes to cells

1. Add DNA:PLUS™ Reagent:Lipofectamine® LTX Reagent complexes from the complexation plate to the corresponding column of a 24-well plate containing your cells of interest (transfection plate). The specific amounts of transfection complexes to be added from the complexation plate to each well of the transfection plate are indicated in the diagram below.

Complexation Plate

	1	2	3	4	5	6
A	Well A1	Well A2	Well A3	Well A4	Well A5	Well A6

Transfection Plate

	1	2	3	4	5	6
A	50 µL	50 µL	50 µL	50 µL	50 µL	50 µL
B	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL
C	150 µL	150 µL	150 µL	150 µL	150 µL	150 µL
D	200 µL	200 µL	200 µL	200 µL	200 µL	200 µL

   The wells of row A contain 250 ng of DNA, increasing progressively by 250 ng in each row, while each column represents a 1X increase in the ratio of lipid to DNA.
2. For no treatment and/or DNA alone controls create a second 24-well plate or do not add transfection complexes to well D6

Incubate cells and assay for gene expression

Return the cells to the incubator, and assay for gene expression in 18–48 hours.

After Optimization

Record the combination of DNA and Lipofectamine® LTX Reagent that provides the optimal conditions for transfection, and use those conditions for future transfections of the cell type used for optimization. There is no need for repeating the optimization protocol unless you switch to a different cell type.

Generating Stable Cell Lines

Passage cells at 1:10 (or higher dilution) into fresh medium 1 day after transfection with either protocol. Add selection medium (if desired) the following day. Visit **www.invitrogen.com/transfection** for cell-specific transfection protocols.

Scaling Up or Down Transfections

To transfect cells in different tissue culture formats, vary the amounts of transfection complex and medium used in proportion to the relative surface area, as shown in the table below **on a per well basis**. Determine the optimal amount required for Lipofectamine® LTX Reagent, plasmid DNA, and PLUS™ Reagent as described in the **Protocol** for 24-well format.

Culture vessel	Surface area per well[1]	Volume plating medium	Relative Surface Area (compared to 24-well)
96-well	0.3 cm^2	100 µL	0.2X
48-well	1.0 cm^2	200 µL	0.4X
24-well	**2 cm^2**	**500 µL**	**1X**
12-well	4 cm^2	1 mL	2X
6-well	10 cm^2	2 mL	5X

[1] Surface areas may vary depending on the manufacturer.

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