

# Lipofectamine™ LTX Reagent

**Size: 1.0 ml**

**Store at +4°C (do not freeze)**

## Description

Lipofectamine™ LTX Reagent is a proprietary, animal-origin free formulation for the transfection of DNA into eukaryotic cells offering the following advantages:

- Highest transfection expression performance with **low cytotoxicity** in many cell types and formats (e.g. 96 well).
- Easy One-Tube protocol for complex formation of Lipofectamine™ LTX Reagent with DNA.
- Reduced cytotoxicity of Lipofectamine™ LTX Reagent allows for use of a greater range of lipid doses, allowing excellent transfection results despite differences in cell density, minor pipetting inaccuracies, and other variations.

## Important Guidelines for Transfection

- We recommend the **One Tube Protocol** (page 2) for most applications. For high-throughput experiments, or if you dispense small amounts of Lipofectamine™ LTX Reagent (< 0.5 µl), we advise pre-diluting the lipid as described in the **High-Throughput Protocol** (page 3).
- 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 (Cat. No. 13778-075).
- For some cell lines, using PLUS™ Reagent (Cat. No. 11514-015) enhances transfection performance.
- Visit [www.invitrogen.com/genedelivery](http://www.invitrogen.com/genedelivery) or contact Technical Service 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) to dilute the 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.

Part No.: 15338.pps

Rev. Date: 1 May 2006

## One Tube Protocol

Use this procedure to transfect DNA into mammalian cells in a **24-well format**. For other formats, see **Scaling Up or Down Transfections** (page 4). All amounts are given on a per well basis. Use this procedure as a starting point; optimize transfections as described in **Optimizing Transfections** (below).

**Note:** Read **Important Guidelines for Transfection** (page 1) before using antibiotics.

- Adherent cells:** One day before transfection, plate cells in 500  $\mu$ l of growth medium so that the cells will be 50-80% confluent at the time of transfection.  
**Suspension cells:** Just prior to preparing complexes plate 200,000-500,000 cells in 500  $\mu$ l of growth medium.
- For each transfection sample**, prepare complexes as follows:
  - Dilute 500 ng plasmid DNA in 100  $\mu$ l Opti-MEM<sup>®</sup> I Reduced Serum Medium without serum. Mix gently.
  - Only if using PLUS<sup>™</sup> Reagent:** Mix PLUS<sup>™</sup> Reagent gently before use, then add 0.5  $\mu$ l PLUS<sup>™</sup> Reagent directly to the diluted DNA. Mix gently and incubate for 5 minutes at room temperature.
  - Mix Lipofectamine<sup>™</sup> LTX Reagent gently before use, then add 1.25  $\mu$ l directly to the diluted DNA. Mix gently.
  - Incubate for 30 minutes at room temperature. Complexes are stable for 6 hours at room temperature.
- Add the ~100  $\mu$ l DNA-Lipofectamine<sup>™</sup> LTX complexes to the well containing cells. Mix gently by rocking the plate back and forth.
- Incubate the cells at 37°C in a CO<sub>2</sub> incubator for 18-48 hours prior to testing for transgene expression. Medium may be changed after 4-6 hours.

## Optimizing Transfections

To obtain the highest transfection performance, vary the amounts of DNA and Lipofectamine<sup>™</sup> LTX as described below for **24-well format** (for other formats, adjust accordingly). Consider varying cell density and using PLUS<sup>™</sup> Reagent.

**Note:** Visit [www.invitrogen.com/genedelivery](http://www.invitrogen.com/genedelivery) for cell-specific transfection protocols.

Cells	DNA	Lipofectamine <sup>™</sup> LTX	PLUS <sup>™</sup> Reagent
Sensitive cells (Hela, HT1080)	250 ng	0.375 $\mu$ l – 1.25 $\mu$ l	0.125 $\mu$ l – 0.5 $\mu$ l
Most cell lines	500 ng	0.75 $\mu$ l – 3.0 $\mu$ l	0.25 $\mu$ l – 1.0 $\mu$ l
	750 ng	1.125 $\mu$ l – 4.5 $\mu$ l	0.375 $\mu$ l – 1.5 $\mu$ l
Suspension and robust cells <sup>1</sup>	1000 ng	1.5 $\mu$ l – 5.0 $\mu$ l	0.5 $\mu$ l – 2.0 $\mu$ l

<sup>1</sup> Examples are MCF7, A549, Jurkat, THP1 and HL60

## High-Throughput Protocol

Use this procedure to transfect DNA into mammalian cells if performing high-throughput transfections, or if dispensing small amounts of Lipofectamine™ LTX Reagent (or PLUS™ Reagent). In this procedure, these reagents are pre-diluted first, and then a larger volume is added to the diluted DNA. Discard diluted reagents after use, as diluted lipid loses activity after 5 minutes. Amounts are for **96-well format**; for other formats, see **Scaling Up or Down Transfections** (page 4). All amounts are given on a per well basis. Use this procedure as a starting point; optimize transfections as described in **Optimizing Transfections** (page 2). **Note:** Read **Important Guidelines for Transfection** (page 1) before using antibiotics.

- 1. Adherent cells:** One day before transfection, plate cells in 100 µl of growth medium so that the cells will be 50-80% confluent at the time of transfection.  
**Suspension cells:** Just prior to preparing complexes plate 40,000-100,000 cells in 100 µl of growth medium without antibiotics.
- 2. For each transfection sample,** prepare complexes as follows:
  - a. Dilute 100 ng plasmid DNA in 10 µl Opti-MEM® I Reduced Serum Medium without serum. Mix gently.
  - b. **Only if using PLUS™ Reagent:** Mix PLUS™ Reagent gently before use, then dilute the appropriate amount (0.1 µl per well) 10-fold in Opti-MEM® I Reduced Serum Medium without serum. Add 1 µl diluted PLUS™ Reagent to diluted DNA. Mix gently and incubate 5 minutes at room temperature.
  - c. Make a master Lipofectamine™ LTX dilution: mix Lipofectamine™ LTX gently, aliquot the appropriate amount (0.25 µl per well). Add to this the appropriate amount of Opti-MEM® I Reduced Serum Medium without serum (10 µl per well). Mix gently. Proceed to step d within **5 minutes**.
  - d. Add 10 µl of diluted Lipofectamine™ LTX to the diluted DNA. Mix gently.
  - e. Incubate for 30 minutes at room temperature. Complexes are stable for 6 hours at room temperature.
- 3.** Add the ~20 µl DNA-Lipofectamine™ LTX complexes to each well containing cells. Mix gently by rocking the plate back and forth.
- 4.** Incubate the cells at 37°C in a CO<sub>2</sub> incubator for 18-48 hours prior to testing for transgene expression. Medium may be changed after 4-6 hours.

## Generating Stable Cell Lines

Passage cells at 1:10 (or higher dilution) into fresh medium 1 day after transfection with either protocol. Add selective medium (if desired) the next day.

## Scaling Up or Down Transfections

To transfect cells in different tissue culture formats, vary the amounts of Lipofectamine™ LTX Reagent, DNA, cells, medium and PLUS™ Reagent used in proportion to the relative surface area, as shown in the table (amounts given on a per well basis).

Culture vessel	Surface area per well <sup>1</sup>	Volume plating medium	Volume dilution medium <sup>2</sup>	DNA	Lipofectamine™ LTX	PLUS™ Reagent
96-well	0.3 cm <sup>2</sup>	100 µl	20 µl	100 ng	0.25 µl	0.1 µl
48-well	1.0 cm <sup>2</sup>	200 µl	40 µl	200 ng	0.5 µl	0.2 µl
24-well	2 cm <sup>2</sup>	500 µl	100 µl	500 ng	1.25 µl	0.5 µl
12-well	4 cm <sup>2</sup>	1 ml	200 µl	1 µg	2.5 µl	1.0 µl
6-well	10 cm <sup>2</sup>	2 ml	500 µl	2.5 µg	6.25 µl	2.5 µl

<sup>1</sup>Surface areas may vary depending on the manufacturer.

<sup>2</sup>If using the **High Throughput Protocol**, use half the amount of dilution medium per dilution, since both Lipofectamine™ LTX and DNA are diluted in this protocol.

## Reverse Transfection

You may perform rapid 96-well plate transfections by plating cells directly into the transfection mix. Prepare complexes in the plate and directly add cells at twice the cell density as in the basic protocol in a 100 µl volume. Cells will adhere as usual in the presence of complexes. Lipid doses must also be optimized, as in most cases more lipid is required for optimal transfection.

## Quality Control

Lipofectamine™ LTX is tested for the following aspects:

- For absence of microbial and fungal contamination with blood agar plates, Sabaraud dextrose agar plates, and fluid thioglycolate medium.
- Functionally by transfection of CHO-K1 cells with a reporter plasmid.

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