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Lipofectamine® LTX and PLUS™ Reagents

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Cat. no.	Lipofectamine® LTX	PLUS™ Reagent	Store
15338-100	1.0 mL	0.85 mL	4°C (do not freeze)
15338-500	15.0 mL	available separately, Cat. no. 11514-015	4°C (do not freeze)

Description

Lipofectamine® LTX Reagent is a proprietary, animal-origin free cationic lipid formulation for transfecting DNA into eukaryotic cells that offers:

- An easy protocol allowing for the highest transfection expression performance with *low cytotoxicity* in many cell types and formats (e.g. 96-well).
- Reduced cytotoxicity of Lipofectamine® LTX Reagent, which allows the use of a greater range of lipid doses, enabling excellent transfection results despite differences in cell density, minor pipetting inaccuracies, and other variations.

Important Guidelines for Transfection

- For best results, optimize DNA and lipid amounts.
- 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).
- Visit **www.lifetech.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) 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.

Intended Use: For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Optimizing Transfections

To obtain the highest transfection performance, perform optimization by using a range of DNA and Lipofectamine® LTX amounts with and without the PLUS™ Reagent as described below for a *24-well format*. For other formats, adjust the amounts according to **Scaling Up or Down Transfections** (page 4). Consider varying the cell density for further optimization.

Note: Visit **www.lifetech.com/transfection** for cell-specific transfection protocols.

Cells	DNA	Lipofectamine® LTX	PLUS™ Reagent
Sensitive cells (HeLa, HT1080)	250 ng	0.375–1.25 µL	0.125–0.5 µL
Most cell lines	500 ng	0.75–3.0 µL	0.25–1.0 µL
	750 ng	1.125–4.5 µL	0.375–1.5 µL
Suspension and robust cells[1]	1000 ng	1.5–5.0 µL	0.5–2.0 µL

[1] Examples are MCF7, A549, Jurkat, THP1 and HL60

Standard Protocol

Transfect DNA into mammalian cells in a *24-well format* after optimizing the reactions as described above. *If using the PLUS™ Reagent, start with a 1:1 ratio of DNA (µg) to PLUS™ Reagent (µL).* All amounts are given on a per-well basis. **Note**: Read **Important Guidelines for Transfection** before using antibiotics.

1. **Adherent cells:** One day before transfection, plate cells in 500 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 100,000–250,000 cells in 500 µL of growth medium.
2. *For each transfection sample,* prepare complexes as follows:
   a. Dilute the optimized amount of plasmid DNA in 100 µL Opti-MEM® I Reduced Serum Medium. Mix thoroughly.
   b. *Only if using PLUS™ Reagent:* Mix PLUS™ Reagent gently before use, add the optimized volume of PLUS™ Reagent directly to the diluted DNA. Mix gently and incubate for 5 minutes at room temperature.
   c. Mix Lipofectamine® LTX gently before use, and add the optimized volume directly to the diluted DNA. Mix thoroughly.
   d. Incubate the mixture for 30 minutes at room temperature. DNA-lipid complexes are stable for 6 hours at room temperature**.**
3. Add ~100 µL DNA-lipid complex (from step 2d of this procedure) dropwise to the well containing cells. Mix gently by rocking the plate back and forth.
4. Incubate the cells at 37°C in a CO_2 incubator for 18–48 hours prior to testing for transgene expression. The medium may be changed after 4–6 hours.

High-Throughput Protocol

Use these guidelines for high-throughput transfections, or if dispensing small amounts of reagents. Pre-dilute the reagents first, and then add this larger volume to the diluted DNA. Discard unused diluted reagents because diluted lipid loses activity after 5 minutes. All amounts are given on a per-well basis for the *96-well format;* for other formats, refer to **Scaling Up or Down Transfections**. *For best results, optimize transfections as described in Optimizing Transfections.*

Note: Review **Important Guidelines for Transfection** 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 20,000–50,000 cells in 100 µL of growth medium without antibiotics.

2. *For each transfection sample,* prepare DNA-lipid complexes as follows:

   a. Dilute the optimized amount of plasmid DNA in 10 µL Opti-MEM® I Reduced Serum Medium. Mix gently.

   b. *Only if using PLUS™ Reagent:* Mix PLUS™ Reagent gently. Prepare a 1:10 dilution of PLUS™ Reagent in Opti-MEM® I Reduced Serum Medium. Add the optimized volume of diluted PLUS™ Reagent to the diluted DNA. Mix gently and incubate the plate for 5 minutes at room temperature.

   c. Mix Lipofectamine® LTX gently. Prepare a "Master Mix" by making a 1:10 dilution of Lipofectamine® LTX in Opti-MEM® I Reduced Serum Medium.

   d. Aliquot the appropriate amount of the "Master Mix" (equivalent to the optimized amount of Lipofectamine® LTX), and bring up the final volume to 10 µL per well with Opti-MEM® I Reduced Serum Medium. Mix gently. *Proceed to the next step (step 2e) within 5 minutes.*

   e. Add the diluted Lipofectamine® LTX to the diluted DNA. Mix gently.

   f. Incubate for 30 minutes at room temperature. DNA-lipid complexes are stable for 6 hours at room temperature**.**

3. Add ~20 µL DNA-Lipofectamine® LTX complexes to each well containing cells. Mix gently by rocking the plate back and forth.

4. Incubate the plate at 37°C in a CO_2 incubator for 18–48 hours and test 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 selection medium (if desired) the following day.

Scaling Up or Down Transfections

To transfect cells in different tissue culture formats, vary the amounts of DNA, Lipofectamine® LTX, PLUS™ Reagent, cells, and medium used in proportion to the relative surface area, as shown in the following table and given on a per well basis. Determine the optimal amount required for Lipofectamine® LTX, plasmid DNA, and PLUS™ Reagent (if used) as described in **Optimizing Transfections** (page 2) for 24-well format.

Culture vessel	Surface area per well[1]	Volume plating medium	Volume dilution medium[2]	Relative Surface Area (compared to 24-well)
96-well	0.3 cm^2	100 µL	20 µL	0.2X
48-well	1.0 cm^2	200 µL	40 µL	0.4X
24-well	2 cm^2	500 µL	100 µL	**1X**
12-well	4 cm^2	1 mL	200 µL	2X
6-well	10 cm^2	2 mL	500 µL	5X

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

[2] Lipofectamine® LTX and DNA are diluted separately in the High Throughput Protocol, each into one half of the total volume of dilution medium.

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 standard protocol in a 100 µL volume. Cells will adhere as usual in the presence of complexes. *Optimize lipid doses because, in most cases, more lipid is required for optimal transfection using this method.*

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