Transfecting Plasmid DNA into NIH3T3 Cells Using Lipofectamine™ LTX Reagent

Introduction

Lipofectamine™ LTX Reagent is a proprietary, animal-origin free formulation for the transfection of DNA into eukaryotic cells with **low cytotoxicity**. This reference provides a recommended procedure to transfect plasmid DNA into NIH3T3 Mouse Embryonic Fibroblasts (ATCC Cat. No. CRL-1658) using Lipofectamine™ LTX Reagent (Cat. No. 15338-100).

Important Guidelines for Transfection

Follow these important guidelines when transfecting DNA into NIH3T3 cells using Lipofectamine™ LTX Reagent:

- The addition of antibiotics to media during transfection may result in cell death, and has not been tested for NIH3T3 cells. If you wish to use antibiotics during transfection, test your conditions thoroughly.
- Maintain the same seeding conditions between experiments. Use low-passage cells; make sure that cells are healthy and greater than 90% viable before transfection.
- Transfection can be performed both in the presence or absence of serum. Test serum-free media for compatibility with Lipofectamine™ LTX Reagent.
- Using PLUS™ Reagent (Cat. No. 11514-015) enhances transfection performance in NIH3T3 cells.
- We recommend Opti-MEM® I Reduced Serum Medium (Cat. No. 31985-062) to dilute the DNA and Lipofectamine™ LTX Reagent before complexing.
- Visit www.invitrogen.com/transfection or contact Technical Service for other specialized transfection protocols (including cell-type specific advice on use of PLUS™ Reagent and antibiotics, and a protocol for vector-based RNAi).
- Lipofectamine™ LTX Reagent performs well with vector-based RNAi experiments. For siRNA and Stealth™ RNAi transfections, we recommend Lipofectamine™ RNAiMAX (Cat. No. 13778-075). Go to www.invitrogen.com/RNAi or contact Technical Service for more information.

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Materials Needed

Have the following reagents on hand before beginning:

- NIH3T3 cells maintained in DMEM supplemented with L-glutamine (Cat. No. 11965-084), 0.1 mM MEM Non-Essential Amino Acids Solution (Cat. No. 11140-050), and 10% Fetal Bovine Serum (Cat. No. 26140-079). Grow cells at 37°C with 5% CO_2.
- Plasmid DNA of interest (100 ng/µl or higher)
- Lipofectamine™ LTX Reagent (store at +4°C until use), and PLUS™ Reagent (if desired; store at 4°C)
- Opti-MEM® I Reduced Serum Medium
- Appropriate tissue culture plates and supplies

Transfection of NIH3T3 Cells

Use this procedure to transfect plasmid DNA into NIH3T3 cells in a **24-well format** (for other formats, see **Scaling Up or Down Transfections**, below). All amounts and volumes are given on a per well basis.

1. The day before transfection, trypsinize and count the cells. Plate 4 x 10^4 cells per well in 0.5 ml of complete growth medium. Cell density should be 50~80% confluent on the day of transfection.
2. For each well of cells to be transfected, dilute 0.5 µg of DNA into 100 µl of Opti-MEM® I Reduced Serum Medium without serum.
3. If using PLUS™ Reagent: Mix PLUS™ Reagent gently before use, then add 0.5 µl PLUS™ Reagent (a 1:1 ratio to DNA) directly to the diluted DNA. Mix gently and incubate for 5-15 minutes at room temperature.
4. For each well of cells, dilute 0.75-2.25 µl of Lipofectamine™ LTX into the above diluted DNA solution, mix gently and incubate for 25 minutes at room temperature to form DNA-Lipofectamine™ LTX complexes.
5. Remove growth medium from cells and replace with 0.5 ml of complete growth medium. Add 100 µl of the DNA-Lipofectamine™ LTX complexes directly to each well containing cells and mix gently by rocking the plate back and forth.
6. Complexes do not have to be removed following transfection. Incubate the cells at 37°C in a CO_2 incubator for 18-24 hours post-transfection before assaying for transgene expression.

Scaling Up or Down Transfections

To transfect NIH3T3 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[1]	Volume plating medium	Cells per well	Volume dilution medium[2]	DNA	Lipofectamine™ LTX Reagent	PLUS™ Reagent
96-well	0.3 cm^2	100 µl	8 x 10^3	20 µl	100 ng	0.15 - 0.45 µl	0.1 µl
48-well	1 cm^2	200 µl	2 x 10^4	40 µl	200 ng	0.3 – 0.9 µl	0.2 µl
24-well	2 cm^2	500 µl	4 x 10^4	100 µl	500 ng	0.75 – 2.25 µl	0.5 µl
12-well	4 cm^2	1 ml	8 x 10^4	200 µl	1 µg	1.5 – 4.5 µl	1.0 µl
6-well	10 cm^2	2 ml	2 x 10^5	500 µl	2.5 µg	3.75 – 11.25 µl	2.5 µl

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

[2] If the volume of Lipofectamine™ LTX Reagent is too small to dispense accurately, and you cannot pool dilutions, predilute Lipofectamine™ LTX Reagent 10-fold in Opti-MEM® I Reduced Serum Medium, and dispense a 10-fold higher amount (should be at least 1.0 µl per well). Discard any unused diluted Lipofectamine™ LTX Reagent.

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