Transfecting Plasmid DNA into HT-29 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 HT-29, human colon adenocarcinoma cells (ATCC No. HTB-38) using Lipofectamine LTX™ Reagent.

Important Guidelines for Transfection

Follow these important guidelines when transfecting HT-29 cells using Lipofectamine LTX™ Reagent:

- Maintain the same seeding conditions between experiments. Use low-passage cells; make sure 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.
- We recommend Opti-MEM® I Reduced Serum Medium (Cat. No. 31985-070) to dilute the DNA Lipofectamine LTX™ Reagent before complexing.
- Using PLUS™ Reagent (Cat. No. 11514-015) enhances transfection performance in HT-29 cells
- Visit www.invitrogen.com/genedelivery or contact Technical Services for other specialized transfection protocols.
- Lipofectamine LTX™ Reagent performs well with vector-based RNAi experiments. For siRNA and Stealth RNAi transfections, we recommend Lipofectamine RNAiMAX. 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:

- HT-29 cells maintained in RPMI Medium 1640 (Cat. No. 21870-076) supplemented with 4 mM L-Glutamine (Cat. No. 25030-081), 10% fetal bovine serum (Cat. No. 16000-044). Grow cells at 37° C with 5% CO_2
- Plasmid DNA of interest
- Lipofectamine LTX™ Reagent (store at +4°C until ready to use) , and PLUS™ Reagent (if desired; store at 4°C)
- Opti-MEM® I Reduced Serum Media
- Appropriate tissue culture plates and supplies

Transfecting HT-29 Cells

Use this procedure to transfect plasmid DNA into HT-29 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 $1.5x10^5$ cells per well in 0.5 ml of complete growth medium. Cell density should be 50-80% confluent on the day of transfection.
2. (Optional) The day of transfection, remove growth medium from cells and replace with 0.5 ml of complete growth medium.
3. For each well of cells to be transfected, dilute 0.75 µg of DNA in 100 µl of Opti-MEM® I Reduced Serum Media without serum.
4. If using PLUS™ Reagent: Mix PLUS™ Reagent gently before use, then add 0.75 µl PLUS™ Reagent (a 1:1 ratio to DNA) directly to the diluted DNA. Mix gently and incubate 5-15 minutes at room temperature.
5. For each well of cells, add 3.75-5.0 µl of Lipofectamine LTX™ Reagent into the above diluted Opti-MEM®:DNA solution, mix gently and incubate 30 minutes at room temperature to form DNA- Lipofectamine LTX™ Reagent complexes.
6. After 30 minute incubation, add 100 µl of the DNA- Lipofectamine LTX™ Reagent complexes directly to each well containing cells and mix gently by rocking the plate back and forth.
7. 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

Culture Vessel	Surface Area per well	Volume Plating Medium	Cells per well	Volume Dilution Medium	DNA	Lipofectamine LTX™ Reagent	PLUS™ Reagent
96-well	0.3 cm^2	100 µl	3.0×10^4	20 µl	150 ng	0.75 – 1.0 µl	0.15 µl
48-well	1 cm^2	200 µl	6.0×10^4	40 µl	300 ng	1.5 – 2.0 µl	0.3 µl
24-well	2 cm^2	500 µl	1.5×10^5	100 µl	750 ng	3.75 – 5 µl	0.75 µl
12-well	4 cm^2	1 ml	3.0×10^5	200 µl	1.5 µg	7.5 – 10 µl	1.5 µl
6-well	10 cm^2	2 ml	7.5×10^5	500 µl	3.75 µg	18.75 – 25 µl	3.75 µl

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