



Transfecting Plasmid DNA into CHO-K1 Cells Using Lipofectamine™ LTX Reagent

Terms and Conditions of Use

Invitrogen Corporation ("Invitrogen") grants to you, its customer, a non-exclusive, non-transferable license to access the Invitrogen transfection protocol applicable to Invitrogen products, as set forth below (the "Protocol"). You acknowledge that the Protocol is protected by copyright. All rights not specifically granted to you are expressly reserved to Invitrogen. You may use the Protocol for personal, educational, or scientific research or professional use, but in no case for a fee. You may not remove, obscure or modify any copyright or proprietary notices, author attribution or any disclaimer contained in the Protocol.

INVITROGEN PROVIDES THE PROTOCOL "AS IS", WITHOUT WARRANTIES OF ANY KIND, EITHER EXPRESS OR IMPLIED, INCLUDING, BUT NOT LIMITED TO, WARRANTIES OF TITLE, OR IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE OR NON-INFRINGEMENT; YOUR USE OF THE PROTOCOL IS AT YOUR OWN RISK; NEITHER INVITROGEN NOR ITS AFFILIATES, EMPLOYEES, OFFICERS OR DIRECTORS SHALL BE LIABLE TO YOU OR ANY THIRD PARTY FOR ANY DIRECT, INDIRECT, INCIDENTAL, SPECIAL, CONSEQUENTIAL OR PUNITIVE DAMAGES ARISING OUT OF THIS LICENSE OR ANY USE OF THE PROTOCOL. Some jurisdictions do not allow the exclusion or limitation of liability for certain damages, so the above limitation may not apply to you to the extent prohibited by such local laws; if so, then Invitrogen's liability for damages hereunder shall not exceed an amount equal to the amounts paid by you hereunder, or one hundred dollars (\$100.00), whichever is less.

Confidentiality

You acknowledge the Protocol is Invitrogen's confidential information and constitutes Invitrogen's proprietary and valuable trade secrets. You agree to take reasonable care to protect the Protocol from disclosure to third parties, except as may be expressly authorized by Invitrogen.

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 CHO-K1 Chinese Hamster Ovary Cells (ATCC Cat. No. CRL-9618) using Lipofectamine™ LTX Reagent (Cat. No. 15338-100).

Important Guidelines for Transfection

Follow these important guidelines when transfecting DNA into CHO-K1 cells using Lipofectamine™ LTX Reagent:

- The addition of antibiotics to media during transfection may result in cell death. 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.
- 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.

Materials Needed

Have the following reagents on hand before beginning:

- CHO-K1 cells maintained in RPMI with L-glutamine (Cat. No. 11875-085) supplemented with 10% fetal bovine serum (Cat. No. 26140-079), and 0.1 mM MEM Non-Essential Amino Acids Solution (Cat. No. 11140-050). Grow cells at 37°C with 5% CO₂.
- Plasmid DNA of interest (100 ng/μl or higher)
- Lipofectamine™ LTX Reagent (store at +4°C until use)
- Opti-MEM® I Reduced Serum Medium
- Appropriate tissue culture plates and supplies

Transfection of CHO-K1 Cells

Use this procedure to transfect plasmid DNA into CHO-K1 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 4×10^4 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. For each well of cells, dilute 0.75-2.75 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.
4. 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.
5. Complexes do not have to be removed following transfection. Incubate the cells at 37°C in a CO₂ incubator for 18-24 hours post-transfection before assaying for transgene expression.

Scaling Up or Down Transfections

To transfect CHO-K1 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 |
|----------------|------------------------------------|-----------------------|-----------------|-------------------------------------|--------|----------------------------|
| 96-well | 0.3 cm ² | 100 μl | 8×10^3 | 20 μl | 100 ng | 0.15 - 0.55 μl |
| 48-well | 1 cm ² | 200 μl | 2×10^4 | 40 μl | 200 ng | 0.3 - 1.1 μl |
| 24-well | 2 cm ² | 500 μl | 4×10^4 | 100 μl | 500 ng | 0.75 - 2.75 μl |
| 12-well | 4 cm ² | 1 ml | 8×10^4 | 200 μl | 1 μg | 1.5 - 5.5 μl |
| 6-well | 10 cm ² | 2 ml | 2×10^5 | 500 μl | 2.5 μg | 3.75 - 13.75 μl |

¹Surface areas may vary depending on the manufacturer.

²If 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.

Purchaser Notification

This product is covered by Limited Use Label License No. 5: Invitrogen Technology (see the Invitrogen catalog or our web-site, www.invitrogen.com). By the use of this product you accept the terms and conditions of the applicable Limited Use Label License.

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

©2006 Invitrogen Corporation. All rights reserved.