

Transfecting Stealth™ RNAi or siRNA into 3T3 L1 Cells Using Lipofectamine™ 2000

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Introduction

Lipofectamine™ 2000 Reagent is a proprietary formulation that facilitates highly efficient delivery of Stealth™ RNAi or short interfering RNA (siRNA) to mammalian cells for RNAi analysis. This reference provides general guidelines and an optimized procedure to transfect Stealth™ RNAi (or siRNA) into mouse 3T3 L1 fibroblast cells (ATCC, Cat. No. CL-173) using Lipofectamine™ 2000 (Cat. No. 11668-027).

Note: While transfection conditions have been optimized to allow highly efficient delivery of Stealth™ RNAi into 3T3 L1 cells, other factors related to the target gene of interest including the transcription rate of the target gene, the stability of the resulting protein, and efficacy of the Stealth™ RNAi sequence chosen can influence the degree of target gene knockdown observed. Take these factors into consideration when designing your RNAi experiment.

Important Guidelines for Transfection

Follow these important guidelines when transfecting Stealth™ RNA (or siRNA) into 3T3 L1 cells using Lipofectamine™ 2000:

1. Use 100 nM Stealth™ RNAi (or siRNA) complexed with 2 µg/ml Lipofectamine™ 2000 (stock solution is 1 mg/ml) for transfection. To increase accuracy and reduce assay variability, we recommend performing transfection **in triplicate** for each sample condition.
2. Transfect 3T3 L1 cells at 60-70% confluence.
3. **Do not add antibiotics** to the medium during transfection as this reduces transfection efficiency and causes cell death.
4. Use Opti-MEM® I Reduced Serum Medium (Cat. No. 31985-062) to dilute Lipofectamine™ 2000 and Stealth™ RNAi (or siRNA) prior to complex formation.

Materials Needed

Have the following reagents on hand before beginning:

- 3T3 L1 cells maintained in D-MEM (Cat. No. 11965-092) supplemented with 10% bovine calf serum (Cat. No. 16170-078), 2 mM glutamine (Cat. No. 25030-149), 1 mM sodium pyruvate (Cat. No. 11360-070) and penicillin/streptomycin (Cat. No. 15070-063)
Note: Use low-passage cells; make sure that cells are healthy and greater than 90% viable before transfection.
- Stealth™ RNAi (or siRNA) of interest (20 µM in annealing buffer)
- Lipofectamine™ 2000 Reagent (store at +4°C until use)
- Opti-MEM® I Reduced Serum Medium (pre-warm to 37°C before use)
- Appropriate tissue culture plates and supplies

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Transfection Procedure

Use this procedure to transfect Stealth™ RNAi (or siRNA) into 3T3 L1 cells using Lipofectamine™ 2000 in a **24-well format**. For other formats, see the table in **Recommended Reagent Amounts and Volumes** for the appropriate reagent amounts to add.

Tip: To reduce well-to-well variability when transfecting multiple replicates (*e.g.* triplicates), proportionally scale up the reagent volumes to form complexes (Step 2), then aliquot an equal volume of complexes into each well.

- One day before transfection, plate 3×10^4 3T3 L1 cells in 400 μ l of growth medium without antibiotics per well. Cells should be 60-70% confluent at the time of transfection.
- For each transfection sample**, prepare Stealth™ RNAi-Lipofectamine™ 2000 complexes as follows:
 - Dilute 50 pmol of Stealth™ RNAi (*i.e.* 2.5 μ l of 20 μ M Stealth™ RNAi) in 50 μ l of Opti-MEM® I Reduced Serum Medium. Mix gently.
 - Mix Lipofectamine™ 2000 gently before use, then dilute 1 μ l in 50 μ l of Opti-MEM® I Reduced Serum Medium. Mix gently and incubate for 15 minutes at room temperature.
 - After the 15-minute incubation, combine the diluted Stealth™ RNAi and the diluted Lipofectamine™ 2000 (total volume ~ 100 μ l). Mix gently and incubate for 15 minutes at room temperature to allow complexes to form (solution may appear cloudy).
- Add the ~100 μ l of Stealth™ RNAi-Lipofectamine™ 2000 complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth.
- Incubate the cells at 37°C in a humidified CO₂ incubator for 16-24 hours as appropriate until you are ready to assay for gene knockdown. It is not necessary to remove the complexes or change the medium; however, growth medium may be replaced after 4-6 hours without loss of transfection activity.

Recommended Reagent Amounts and Volumes

To transfect 3T3 L1 cells in different tissue culture formats, vary the amounts of Stealth™ RNAi (or siRNA), Lipofectamine™ 2000, cells, and medium used in proportion to the relative surface area, as shown in the table. **Note:** 20 μ M Stealth™ RNAi or siRNA = 20 pmol/ μ l.

Culture vessel	Relative surface area (vs. 24-well)	Cells plated per well	Volume of plating medium	Stealth™ RNAi (pmol) in media volume (μ l)	Lipofectamine™ 2000 (μ l) in media volume (μ l)
48-well	0.4	1.5×10^4	200 μ l	20 pmol in 25 μ l	0.4 μ l in 25 μ l
24-well	1	3×10^4	400 μ l	50 pmol in 50 μ l	1 μ l in 50 μ l
6-well	5	1.5×10^5	2 ml	250 pmol in 250 μ l	5 μ l in 250 μ l

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