

Reverse Transfection of Stealth™ RNAi or siRNA using Lipofectamine™ 2000 in a 96 well plate format

Introduction

Lipofectamine™ 2000 Reagent is a proprietary formulation that facilitates highly efficient delivery of Stealth™ RNA molecules or short interfering RNA (siRNA) to mammalian cells for RNAi analysis (1, 2). Here, we describe reverse transfection using Lipofectamine™ 2000. Reverse transfection is a method for high throughput (HTP) transfection of multiple siRNA or Stealth™ RNAi duplexes, and is well suited in combination with RNAi duplexes pre-dispensed in 96-wells plates, such as the Stealth™ RNAi Human Collections (available through our website, www.invitrogen.com).

Reverse transfection combines RNA, transfection reagent, and cells in an altered sequence compared to traditional Lipofectamine™ 2000 transfection protocols. In short, a different RNAi molecule is put in each well prior to transfection and combined with diluted Lipofectamine™ 2000 to form complexes in each well. Cells are added directly to the Lipofectamine™ 2000-RNA complexes and transfection occurs while cells are attaching to the well.

General Guidelines for Transfection

Follow these general guidelines when using Lipofectamine™ 2000 to reverse transfect siRNA or Stealth™ RNAi duplexes into mammalian cells.

- Use low-passage cells, and make sure that cells are healthy and greater than 90% viable before transfection. This protocol is for use with adherent cells, as transfection efficiency for suspension cells generally is lower and may need optimization.
- **Do not add antibiotics to the medium during transfection** as this reduces transfection efficiency and causes cell death. For optimal results, use Opti-MEM® I Reduced Serum Medium to dilute Lipofectamine™ 2000, DNA, and dsRNA oligomers prior to complex formation.
- To increase accuracy and reduce assay variability, we recommend performing **triplicate** transfections for each sample condition.

Materials Needed

You should have the following materials on hand before beginning:

- Mammalian cell line cultured in the appropriate growth medium
- Stealth™ RNAi or siRNA of interest (20 µM stock in 1X RNA Annealing/Dilution Buffer)
- RNAi controls
- Lipofectamine™ 2000 Reagent (Catalog nos. 11668-027 or 11668-019; store at +4°C and mix gently before use)
- Opti-MEM® I Reduced Serum Medium (pre-warmed; Catalog nos. 31985-062 or 31985-070)
- 96-well tissue culture plates and tissue culture supplies

Transfection Procedure

Use this procedure to reverse transfect your siRNA or Stealth™ RNAi duplexes into mammalian cells in 96-wells plates using Lipofectamine™ 2000. The complexes are prepared inside the wells, after which cells and medium are added. Remember to include the proper positive and negative controls in your experiment.

1. **For each well to be transfected**, prepare DNA-RNAi molecule-Lipofectamine™ 2000 complexes as follows.
 - a. Mix Lipofectamine™ 2000 gently before use, then dilute 0.25 µl Lipofectamine™ 2000 in 25 µl Opti-MEM® I Medium without serum in a separate vessel. Mix gently and incubate for 5 minutes at room temperature.
 - b. Dilute 15 pmol siRNA or Stealth™ RNAi in 25 µl Opti-MEM® I Medium without serum in the well of the tissue culture plate. Mix gently.
 - c. After the 5 minute incubation, add the diluted Lipofectamine™ 2000 to the wells with the diluted RNAi molecules. Mix gently and incubate for 15 minutes at room temperature to allow complex formation to occur. The solution may appear cloudy, but this will not impede the transfection.
2. Add 100 µl complete growth medium **without antibiotics** with 20,000 cells to each well containing RNAi molecule-Lipofectamine™ 2000 complexes. This gives a final volume of 150 µl and a final RNA concentration of 100 nM. Mix gently by rocking the plate back and forth.
3. Incubate the cells at 37°C in a CO₂ incubator until you are ready to harvest cells and assay for your target gene.

We recommend harvesting cells 24-48 hours after transfection to assay for target gene knockdown.

Transfection Optimization.

- 0.25 μ l Lipofectamine™ 2000 per well is recommended as a starting point but optimization of transfection conditions may be required. A range of 0.125 μ l to 1 μ l is recommended.
- Note that for highly potent RNAi molecules (*i.e.* RNAi molecules inducing > 90% target knockdown), the amount of dsRNA required to obtain effective knockdown may be less than the amounts specified. This needs to be determined empirically for each cell line.

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References

1. Gitlin, L., Karelsky, S., and Andino, R. (2002) *Nature* 418, 430-434.
2. Yu, J.Y., DeRuiter, S.L., and Turner, D.L. (2002) *Proc. Natl. Acad. Sci. USA* 99, 6047-6052.

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