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Cotransfecting Plasmid DNA and RNAi into Mammalian Cells Using Lipofectamine™ 2000

Introduction

Lipofectamine™ 2000 Reagent is a proprietary formulation that facilitates highly efficient delivery of Stealth™ RNA molecules, short interfering RNA (siRNA) or plasmid DNA to mammalian cells for RNAi analysis (1, 2). This reference provides general guidelines and a procedure to cotransfect plasmid DNA and an RNAi molecule (*i.e.* Stealth™ RNAi, siRNA, shRNA plasmid or miRNA plasmid) into mammalian cells using Lipofectamine™ 2000 Reagent.

General Guidelines for Transfection

Follow these general guidelines when using Lipofectamine™ 2000 to cotransfect your plasmid DNA and the RNAi molecule of interest into mammalian cells.

- Use low-passage cells, and make sure that cells are healthy and greater than 90% viable before transfection.
- **Transfect cells at 80-90% confluence.**
- **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.
- Stealth™ RNAi duplexes or siRNA are generally supplied as a 20 µM stock solution. If you are performing transfection in a format smaller than a 6-well dish (*e.g.* 24-well format), we recommend diluting the 20 µM stock solution 10- to 20-fold in DEPC-treated water to prepare a 1-2 µM stock solution, as appropriate. Use the 1-2 µM stock solution for transfection. Store the 2 µM stock solution at -20°C.

  Example: To prepare a 2 µM stock solution, dilute 2 µl of the 20 µM siRNA or Stealth™ RNAi stock solution in 18 µl of DEPC-treated water.
- 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
- Plasmid DNA (0.1-3.0 µg/µl in sterile water or TE Buffer, pH 8.0)
- Stealth™ RNAi or siRNA of interest (20 µM stock in 1X RNA Annealing/Dilution Buffer) or shRNA or miRNA expression plasmids of interest (0.1-3.0 µg/µl in sterile water or TE Buffer, pH 8.0)
- 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)
- Appropriate tissue culture plates and supplies

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For technical support, contact tech_service@invitrogen.com.

Transfection Procedure

Use this procedure to cotransfect your plasmid DNA and the RNAi molecule into mammalian cells using Lipofectamine™ 2000. Refer to the table in **Suggested Reagent Amounts and Volumes** for the appropriate reagent amounts and volumes to add for different tissue culture formats. Remember to include the proper positive and negative controls in your experiment.

1. One day before transfection, plate cells in the appropriate amount of growth medium **without antibiotics** such that they will be 80-90% confluent at the time of transfection.
2. **For each transfection sample,** prepare DNA-RNAi molecule-Lipofectamine™ 2000 complexes as follows.
   a. Dilute the DNA and RNAi molecule in the appropriate amount of Opti-MEM® I Medium without serum. Mix gently.
   b. Mix Lipofectamine™ 2000 gently before use, then dilute the appropriate amount in Opti-MEM® I Medium without serum. Mix gently and incubate for 5 minutes at room temperature.
   c. After the 5 minute incubation, combine the diluted DNA and RNAi molecule with the diluted Lipofectamine™ 2000. Mix gently and incubate for 20 minutes at room temperature to allow complex formation to occur. The solution may appear cloudy, but this will not impede the transfection.
3. Add the DNA-RNAi molecule-Lipofectamine™ 2000 complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth.
4. Incubate the cells at 37°C in a CO_2 incubator until you are ready to harvest cells and assay for your target gene. Removal of complexes or media change is not required; however, growth medium may be replaced after 4-6 hours without loss of transfection activity.

Tip: We recommend harvesting cells 24-48 hours after transfection.

Suggested Reagent Amounts and Volumes

The table below lists the range of recommended reagent amounts and volumes to use to transfect cells in various tissue culture formats. As a starting point, use an amount of plasmid DNA (see column 4), dsRNA or RNAi vector DNA (see column 5), and Lipofectamine™ 2000 (see column 7) that falls around the mid-point of the recommended range, then optimize conditions for your cell line by varying reagent amounts within the recommended range. If you wish to perform transfection in 96-well format, see the additional guidelines in **Guidelines for Transfection in 96-Well Format**, next page.

Example: We typically use 150 ng of plasmid DNA, 5 pmol of Stealth™ RNAi, and 1 µl of Lipofectamine™ 2000 to transfect GripTite™ 293 MSR cells in 24-well format.

Tip: 20 µM dsRNA (*i.e.* siRNA or Stealth™ RNAi) = 20 pmol/µl.

Culture Vessel	Relative Surface Area (vs. 24-well)	Volume of Plating Medium	Plasmid DNA (ng)	dsRNA (pmol)/ RNAi vector (ng)[1]	DNA/RNA Dilution Volume (µl)[2]	Lipid (µl) and Dilution Volume (µl)
96-well	0.2	100 µl	10-100 ng	0.1-1 pmol/150-300 ng	25 µl	0.2-0.5 µl in 25 µl
48-well	0.4	200 µl	50-100 ng	0.5-5 pmol/150-300 ng	25 µl	0.3-0.8 µl in 25 µl
24-well	1	500 µl	100-200 ng	1-10 pmol/300-600 ng	50 µl	0.5-1.5 µl in 50 µl
6-well	5	2 ml	500-1000 ng	5-50 pmol/1.5-3 µg	250 µl	2.5-6 µl in 250 µl

[1]dsRNA = siRNA or Stealth™ RNAi; RNAi vector = shRNA-containing plasmid or miRNA-containing plasmid

[2]Dilute the plasmid DNA **and** the dsRNA or shRNA DNA into this volume of Opti-MEM® I.

Potent RNAi Molecules

Note that for highly potent RNAi molecules (*i.e.* RNAi molecules inducing > 90% target knockdown), the amount of dsRNA or RNAi vector required to obtain effective knockdown may be less than the amounts specified in the table above (see column 5). This needs to be determined empirically for each cell line.

Guidelines for Transfection in 96-Well Format

You may perform the screening experiment in 96-well format, if desired. Note that in this format, the results obtained from the screening experiment are much more sensitive to well-to-well variability caused by differences in cell density, transfection efficiency, and reagent amounts used. If you are transfecting cells in 96-well format, significant optimization of transfection conditions may be required. Follow the guidelines below to cotransfect mammalian cells in 96-well format:

- To address potential problems caused by well-to-well variability, we recommend performing more replicates for each sample condition; **we generally transfect each sample into 6-7 individual wells**.
- When plating cells, make sure that cells are evenly distributed over the surface of each well. As with the other tissue culture formats, transfect cells at 80-90% confluence.
- Use the range of recommended reagent amounts and volumes listed in the table above and optimize accordingly.
- We recommend harvesting cells and assaying for your target gene 24 hours after transfection.

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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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