Transfecting siRNA into Mammalian Cells Using Lipofectamine™ 2000

Introduction

Lipofectamine™ 2000 has been used successfully to transfect short interfering RNAs (siRNA) into mammalian cells for RNA interference (RNAi) studies (Gitlin *et al.*, 2002; Yu *et al.*, 2002). This reference provides general guidelines and procedures to transfect siRNA into mammalian cells using Lipofectamine™ 2000. Suggested transfection conditions are provided for a number of mammalian cell lines as a starting point. If you are using these cell lines or another mammalian cell line, we recommend optimizing transfection conditions to obtain the best results for your target gene and siRNA.

Factors Affecting Gene Knockdown Levels

A number of factors can influence the degree to which expression of a gene of interest is reduced (*i.e.* gene knockdown) in an RNAi experiment including:

- Transfection efficiency
- Transcription rate of the gene of interest
- Protein stability
- Efficacy of the particular siRNA sequence chosen
- Growth characteristics of your mammalian cell line

Take these factors into account when designing your transfection and RNAi experiments.

General Guidelines

Follow these general guidelines when performing siRNA transfection into mammalian cells:

1. **Transfect cells when they are 30-50% confluent.** Gene knockdown levels are generally assayed at a minimum of 24-72 hours following transfection. Transfecting cells at a lower density allows a longer interval between transfection and assay time, and minimizes the loss of cell viability due to cell overgrowth. Depending on the nature of the target gene, higher cell densities may be suitable with optimization of conditions.
2. **Do not add antibiotics** to media during transfection as this will cause cell death.
3. For optimal results, use Opti-MEM® I Reduced Serum Medium (Catalog no. 31985-062) to dilute Lipofectamine™ 2000 prior to complexing with siRNA.

Materials Needed

Have the following reagents on hand before beginning:

- Mammalian cell line of interest (make sure that cells are healthy and greater than 90% viable before transfection)
- siRNA of interest (20 pmol/µl)
- Lipofectamine™ 2000 (store at +4°C until use)
- Opti-MEM® I Reduced Serum Medium (Invitrogen, Catalog no. 31985-062; pre-warmed)
- 24-well tissue culture plates and other tissue culture supplies

continued on next page

Transfecting siRNA into Mammalian Cells Using Lipofectamine™ 2000, continued

Transfection Procedure

Use the following procedure to transfect mammalian cells in a **24-well format**. If you wish to transfect cells in other tissue culture formats, you will need to determine the optimal conditions to use for your mammalian cell line.

If you are transfecting HEK 293, BHK, CHO-K1, or A549 cells, see the table below for suggested transfection conditions. **Note:** In RNAi studies using these conditions, >80% knockdown of a stably integrated reporter gene or an endogenous gene was observed by 24-48 hours after transfection.

Cell Line*	Cell Density (cells/well)	Amount of Lipofectamine™ 2000	Amount of siRNA
HEK 293	1×10^5	1 µl	20 pmol
BHK	1.5×10^4	1 µl	20 pmol
CHO-K1	4×10^4	1 µl	20 pmol
A549	1.5×10^4	1 µl	20 pmol

1. One day before transfection, plate cells in 0.5 ml of growth medium without antibiotics so that they will be 30-50% confluent at the time of transfection.
2. **For each transfection sample**, prepare siRNA:Lipofectamine™ 2000 complexes as follows:
   a. Dilute the appropriate amount of siRNA in 50 µl of Opti-MEM® I Reduced Serum Medium without serum (or other medium without serum). Mix gently.
   b. Mix Lipofectamine™ 2000 gently before use, then dilute the appropriate amount in 50 µl of Opti-MEM® I Medium (or other medium without serum). Mix gently and incubate for 5 minutes at room temperature. **Note:** Combine the diluted Lipofectamine™ 2000 with the diluted siRNA within 30 minutes. Longer incubation times may decrease activity. If D-MEM is used as a diluent for the Lipofectamine™ 2000, mix with the diluted siRNA within 5 minutes.
   c. After the 5 minute incubation, combine the diluted siRNA with the diluted Lipofectamine™ 2000 (total volume is 100 µl). Mix gently and incubate for 20 minutes at room temperature to allow the siRNA:Lipofectamine™ 2000 complexes to form.
3. Add the 100 µl of siRNA:Lipofectamine™ 2000 complexes to each well. Mix gently by rocking the plate back and forth.
4. Incubate the cells at 37°C in a CO_2 incubator for 24-72 hours until they are ready to assay for gene knockdown. It is generally 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.

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Transfecting siRNA into Mammalian Cells Using Lipofectamine™ 2000, continued

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References

Gitlin, L., Karelsky, S., and Andino, R. (2002). Short Interfering RNA Confers Intracellular Antiviral Immunity in Human Cells. Nature *418*, 430-434.

Yu, J. Y., DeRuiter, S. L., and Turner, D. L. (2002). RNA Interference by Expression of Short-interfering RNAs and Hairpin RNAs in Mammalian Cells. Proc. Nat. Acad. Sci. USA *99*, 6047-6052.