siRNA Lipofection Protocol

using Lipofectamine™ RNAiMAX Transfection Reagent

Small interfering RNA (siRNA) plays a critical role in RNA interference (RNAi)-based research and therapeutic development. After binding with the RNA-induced silencing complex (RISC), siRNA specifically binds to the target mRNA and cleaves it, leading to the specific degradation of the target mRNA and thus achieving gene silencing.

Genscript can provide customers with both conventionally chemically synthesized siRNA and modified siRNA according to their needs, with over 200 different modification methods available.

This protocol provides a step-by-step guide for transfecting cells with small interfering RNA (siRNA) via lipofection.

Note: This protocol has been optimized for use with **Lipofectamine™ RNAiMAX**. If using an alternative transfection reagent or method, transfection conditions—such as siRNA and reagent concentrations, incubation times, and cell confluency—must be carefully optimized to achieve efficient gene silencing and low cytotoxicity. Please refer to the corresponding reagent or instrument manuals as relevant.

Required Material

1. Lipofectamine™ RNAiMAX Transfection Reagent (Thermo Fisher Scientific, 13778150)
2. siRNA, lyophilized powder (GenScript, SC1518-RNAi)
3. Opti-MEM™ I Reduced Serum Medium (Thermo Fisher Scientific, 31985062)
4. Endotoxin-free, RNase-free water（Thermo Fisher Scientific, 10977015）
5. 15 mL centrifuge tubes (Corning, 430790)
6. 10 cm cell culture dish (Corning, 430167)
7. 48-well cell culture plate (Corning, 3548)
8. 1.5 mL EP tubes (Axygen, MCT-150-C-S)

General Guidelines

siRNA Storage Conditions

For siRNA in lyophilized powder form, please store at -20°C to -80°C upon receipt. The lyophilized siRNA powder can be stably stored for up to one year.

Before use, centrifuge the lyophilized powder at 12,000 rpm for 1 minute to ensure the siRNA sample is at the bottom of the tube. Prepare a 50 μM siRNA storage solution according to Table 1 below using

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sterile, endotoxin-free, RNase-free water. Gently add the solvent along the tube wall and allow it to dissolve at room temperature for 10-30 minutes, with occasional vortexing to facilitate dissolution. Aliquot the solution according to experimental needs and store any aliquots that will not be immediately used at -20°C to -80°C. Avoid repeated freeze-thaw cycles (no more than 5 times).

Table 1: Preparation of 50 µM siRNA Storage Solution

Reagents	Dosages	Dosages	Dosages
siRNA	2 nmol	5 nmol	10 nmol
Amount Volume of Sterile, Endotoxin-free, RNase-free Water	40 µl	100 µl	200 µl

Precautions

1. If the siRNA is provided in lyophilized powder form, the appearance of the product may vary due to various factors affecting the crystalline morphology. This is a normal phenomenon and does not affect its use.
2. During the experiment, keep the siRNA on ice as much as possible, and store it at -20°C to -80°C immediately after use.
3. Ensure that the experimental area is kept clean. Wear clean masks and gloves while handling. All consumables such as pipette tips and centrifuge tubes used in the experiment must be sterile, endotoxin-free, and RNase-free.
4. All related reagents should be stored and used according to the supplier's instructions.

Lipofection Protocol

The following guidance outlines steps for lipofection of siRNA using Lipofectamine™ RNAiMAX in a 48-well plate, with a working siRNA concentration of 25 nM. For reagent amounts for other vessel specifications, please refer to Table 2 below.

1. Cell Seeding

- Adherent Cells: 18-24 hours before the transfection experiment, digest the adherent cells from a 10 cm dish and resuspend them in a 15 mL centrifuge tube. Take a portion of the cell suspension and count the viable cells using Trypan Blue. Add an appropriate number of cells to 300 µL of complete cell culture medium (ensuring a cell density of 30%-50% at the time of transfection). For example, for HepG2 cells, seed 2-4 × 10^4 cells into each well of a 48-well plate containing 270 µL of complete culture medium.

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- Suspension Cells: On the day of the transfection experiment, add an appropriate number of cells to 270 µL of the antibiotic-free complete cell culture medium (ensuring the cells are not overgrown and are preferably in the logarithmic growth phase, with a cell density of 30%-50%). For example, for HeLa cells, seed 4-8 × 10^4 cells into each well of a 48-well plate containing 270 µL of complete culture medium.

Note: Adjust the seeding density based on the cell doubling time, growth morphology, cytotoxicity response to the transfection reagent, and the detection time of knockdown efficiency. Both high passage numbers and high cell densities can affect transfection efficiency. It is generally recommended to use cells within 20 passages for transfection. Additionally, the seeding density should be such that the cells have not reached full confluence at the time of knockdown efficiency detection.

2. Preparation of Transfection Complex

On the day of transfection, prepare two sterile, endotoxin-free, and nuclease-free 1.5 mL EP tubes for each transfection sample. Label them as tube A and tube B. Then, prepare the transfection complex according to the following steps:

1) In tube A: add 7.5 pmol siRNA, 15 µL Opti-MEM™ I Reduced Serum Medium and mix well.

2) In tube B: add 15 µL Opti-MEM, 0.9 µL Lipofectamine™ RNAiMAX Transfection Reagent and mix well.

Note:

- The optimal working concentration of siRNA and the amount of transfection reagent may vary depending on cell type, target, and experimental purpose. It is recommended to conduct preliminary experiments to optimize the working concentration of siRNA and the amount of transfection reagent. The optimal siRNA working concentration can be optimized by setting up a siRNA concentration gradient, typically ranging from 1 nM to 100 nM.
- It is recommended to optimize the transfection reagent amount by setting up a RNAiMAX gradient. Using a 48-well plate as an example, the amounts generally range from 0.5 µL to 2 µL.
- Do not add serum during the preparation of the transfection complex. It is recommended to use Opti-MEM to dilute siRNA and the transfection reagent. The presence of serum will not affect the transfection efficiency of the complex when subsequently added to the cells.

3) Add the solution from tube A to tube B, mix well, and incubate at room temperature for 5-10 minutes to form the transfection complex.

Note: Do not shake after incubation. It is recommended to keep the transfection complex at room temperature for no longer than 30 minutes.

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3. Cell Transfection

1) During the incubation of the transfection complex, replace the culture medium in the 48-well plate with 270 µL of fresh antibiotic-free medium per well (this step is only required for adherent cells).

2) Add 30 µL of the transfection complex dropwise to each well of the 48-well plate, resulting in a final volume of 300 µL and a final siRNA concentration of 25 nM. Gently shake the plate to mix well after adding the transfection complex.

Note: To minimize the effects of cell plating uniformity, transfection reagent volume, and cell transfection efficiency, and to improve the repeatability and reliability of experimental results, it is generally recommended to set up 2-3 replicates for each transfection sample.

3) Place the culture plate in a 37°C CO_2 incubator for 24-96 hours. Samples can be collected within 24-72 hours to assess gene silencing levels (mRNA; via qRT-PCR or other preferred method), or within 48-96 hours to assess protein levels (via Western Blot, ELISA, antibody staining, or other preferred method.)

Note: The specific incubation time can be adjusted according to experimental needs.

Table 2: Recommended Transfection Conditions for siRNA in Different Culture Vessels

Culture Vessel	Volume of Cell Culture Medium (µL)	Volume of Transfection Complex (µL) (Tube A+B)	Amount of siRNA in Transfection Complex (pmol)	Amount of RNAiMAX in Transfection Complex (µL)
96-well plate	90	10	2.5	0.3
48-well plate	270	30	7.5	0.9
24-well plate	450	50	12.5	1.5
12-well plate	900	100	25	3
6-well plate	2250	250	62.5	7.5
60mm	3600	400	100	12

Note: The working concentration of siRNA during cell transfection was determined to be 25 nM. However, the optimal working concentration of siRNA and the amount of transfection reagent may vary depending on cell type, target, and experimental purpose. It is recommended to conduct preliminary experiments to optimize the working concentration of siRNA and the amount of transfection reagent.

Troubleshooting Guide

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Problem	Possible Cause(s)	Recommended Solution
Low gene knock down efficiency	**Low target gene mRNA levels**	It is recommended to measure the mRNA levels of the target gene. For RNAi experiments, the gene's Ct value in the experimental cell line should generally be less than 30. If the Ct value is higher, consider switching to another cell line.
	Inappropriate siRNA concentration	To achieve optimal gene knockdown, siRNA must reach a certain concentration. It is advisable to start with a concentration of 25 nM in preliminary experiments. Alternatively, based on the target gene's mRNA levels and the transfection efficiency in the cell line, you can test a gradient of siRNA concentrations to identify the most suitable one.
	Low siRNA transfection efficiency	1) Optimize transfection conditions using a positive control. This includes adjusting the transfection time, cell seeding density, and the amount of transfection reagent used. 2) If necessary, try transfecting other cell lines, such as HEK293T. 3) If transfecting other cell lines is not feasible, consider using different transfection reagents or alternative transfection methods, such as electroporation.
	Suboptimal siRNA design sequence	If gene knockdown efficiency remains suboptimal despite adequate target gene expression and transfection efficiency in the cell line, it is recommended to use different siRNAs targeting the same gene.
	Note: if none of the recommendations above effectively address low knock down efficiency issues, and 4-5 different siRNAs have been tested without achieving satisfactory gene knock down, it is advisable to review the overall experimental approach and consider switching to a different cell line.	

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