siRNA USER GUIDE

RN：R10043.8

Product Description

The siRNA provided by RiboBio is ready-to-use, chemically synthesized and double-stranded small-molecule RNA.

Shipping & Storage Conditions

The product is shipped as **lyophilized powder** at **room temperature**. After receiving the product, please store it **at -20 ℃ ~ -80 ℃.** Under this condition, the lyophilized powder is stable for one year. Before use, please briefly centrifuge the product, use the **RNase-free Water** to prepare **20μM** stock solution and store it at -20 ℃~-80 ℃. **Please avoid repeated freezing and thawing (no more than 5 times).**

Table 1. Reference for 20μM siRNA stock solution

siRNA	5 nmol	20 nmol
RNase-free Water	250 μl	1000 μl

Before Use

The product is provided in the form of lyophilized powder. Due to many factors affecting the crystal form, the appearance of the product is somewhat different, and even may not be seen with naked eyes. This is a normal phenomenon.

To avoid degradation caused by external factors (including enzymes, extreme pH or temperature conditions, etc.), please strictly follow the protocols of all RNA experiments. During the experiment, the product is best placed on ice. After use, please store it carefully at -20 ℃~-80 ℃.

Cell Experiment Instructions

In order to ensure the reliability of the experiment, reduce the difference between wells caused by cell density, reagent dosage, transfection efficiency and other factors, it is generally recommended that:

1) At least set 3 duplicate wells for each sample in the transfection experiment;
2) When plating cells, the cell quantity of each well should be as consistent as possible and

evenly distributed on each surface.

1. Transfection concentration:

The optimal working concentration of siRNA varies with different cell types and research purposes. The initial transfection concentration of siRNA recommended by RiboBio is 50nM, and the best time window for detection is 24-72h after transfection. Generally, the optimal transfection efficiency is optimized by setting the time gradient and concentration gradient. The siRNA concentration is recommended to be between 5 to 100nM.

2. Transfection steps:

Transfect cells with siRNA and riboFECT™ CP Reagent in a 24-well plate at a transfection concentration of 50 nM. For other culture vessels, please refer to Table 2. For other transfection reagents, please refer to the corresponding instructions.

1) Cell Preparation

a. Adherent cells: Take Hela cells as an example. Plate 1×10^5 ~ 5×10^5 cells to a 24-well plate containing appropriate amount of complete medium. Cell density is recommended to be 30-50% confluent on the day of transfection.

b. Suspended cells: Take THP-1 cells as an example. Plate 1×10^5 ~ 5 x 10^5 cells to a 24-well plate containing appropriate amounts of complete medium.

Note:

① The growth rate varies among different cells types, thus the optimal number of cells plated and cell density depends on cell types, culture time and experimental purposes;

② The number of cells plated per well should be as similar as possible to make the cells evenly distribute;

③ Due to its own characteristics, suspended cells are relatively difficult to transfect, and transfection conditions need to be specifically optimized to improve transfection efficiency. Electroporation could be used if necessary.

2) Transfection

For each transfection sample, please follow the steps below:

a. Dilute siRNA: Dilute 1.25 μl of 20 μM siRNA stock solution (v3) with 30 μl of 1X riboFECT™ CP Buffer (v2), mix gently.

b. Prepare mixed solution: Add 3 μl of riboFECT™ CP Reagent (v4), mix gently by pipetting, and incubate for 0-15 min at room temperature.

Note:

①Do not oscillate. The solution may be turbid, but it will not affect the transfection;

②The mixture can be left at room temperature, but should not exceed 24h.

c. Add the riboFECT™ CP transfection complex to an appropriate amount of complete medium without penicillin and streptomycin (v1), mix gently.

Note: The riboFECT™ CP transfection complex added to the original cell culture medium generally does not need to be removed or replaced, but can be optimized for specific experimental conditions.

d. (Optional) Perform other necessary special treatments (such as drug dosing).

e. Incubate the plate in a CO_2 incubator at 37 ℃ for 24 to 96 hours (culture time depends on the purpose of the experiment).

Table 2 Dosage Reference for transfecting siRNA with riboFECT™ CP

v1: cell culture medium; **v2**: 1X riboFECT™ CP Buffer; **v3**: 20μM siRNA; **v4**: riboFECT™ CP Reagent

	siRNA final concentration	Volume per well	cell culture medium	Buffer (v2)	SiRNA (v3)	Reagent (v4)
96-well	100nM	100μl	92.90μl	6μl	0.5μl	0.6μl
	50nM	100μl	93.15μl	6μl	0.25μl	0.6μl
	30nM	100μl	93.25μl	6μl	0.15μl	0.6μl
	20nM	100μl	93.30μl	6μl	0.1μl	0.6μl
	10nM	100μl	93.35μl	6μl	0.05μl	0.6μl
24-well	100nM	500μl	464.50μl	30μl	2.5μl	3μl
*	50nM	500μl	465.75μl	30μl	1.25μl	3μl
	30nM	500μl	466.25μl	30μl	0.75μl	3μl
	20nM	500μl	466.50μl	30μl	0.5μl	3μl
	10nM	500μl	466.75μl	30μl	0.25μl	3μl
12-well	100nM	1ml	929.00μl	60μl	5μl	6μl
	50nM	1ml	931.50μl	60μl	2.5μl	6μl
	30nM	1ml	932.50μl	60μl	1.5μl	6μl
	20nM	1ml	933.00μl	60μl	1μl	6μl
	10nM	1ml	933.50μl	60μl	0.5μl	6μl
6-well	100nM	2ml	1858.00μl	120μl	10μl	12μl
	50nM	2ml	1863.00μl	120μl	5μl	12μl
	30nM	2ml	1865.00μl	120μl	3μl	12μl
	20nM	2ml	1866.00μl	120μl	2μl	12μl
	10nM	2ml	1867.00μl	120μl	1μl	12μl

*: Volume example for reference. Further optimization is required for some cell types.

3. Effect Evaluation:

Evaluation of the siRNA silencing effect can be performed between 24 to 72 hours after

transfection. The optimal detection time depends on cell type, transfection reagent and detection purpose.

a. **Detection of RNA level (standard of RNAi silencing effect evaluation):** The principal of siRNA silencing is to target and shear the RNA molecule of the gene of interest under the action of RISC system. Therefore, the RNA level of the target gene directly indicates the effect of siRNA silencing, and the specific inhibition efficiency can be calculated through qRT-PCR. Generally, the expression of the RNA is significantly decreased 24 to 72 hours after transfection of siRNA.

Note: The quality of primer design is important.

b. **Detection of protein level:** For protein-coding genes, the corresponding protein levels should also be tested to determine if the protein expression level of interest is successfully down-regulated. Since protein expression level is affected by a number of factors, there are sometimes cases where RNA levels are successfully down-regulated but protein levels are not significantly altered. If the protein expression level does not decrease significantly, the expression level of the mRNA should be carefully examined to determine the cause of the unsatisfactory RNAi.

c. **Functional screening:** Directly screen the cell function via EdU cell proliferation and EdUTP cell apoptosis assays to assess the effect of siRNA silencing.

Animal Experiment Instructions

Animal Model: Select the appropriate animal model according to the experiment purpose (Table 3).

Modification: Due to the requirement of high stability of siRNA in animal experiments, siRNA modified with Chol, OMe, PS is commonly used (although unmodified siRNA can also be used). After years of development, RiboBio has developed a 5'Chol + 2'OMe modification siRNA suitable for animal experiments.

Drug administration: topical administration is the most direct method with high efficiency. In this case, siRNA is required in small dosage and quickly absorbed. It is suitable for superficial organs and tissues, including eyes, muscles, subcutaneous tissue, etc.

Systemic administration is suitable for some targets which cannot be reached by topical administration, such as viscera, organs (including heart, Liver, spleen, lung, kidney, etc.) and some scattered targets (such as lymphocytes, metastatic tumor cells, etc.).

Table 3 Reference for Animal siRNA experiment Method

Reference	Animal	Tissue	Modification	Doses	Dosing Frequency

Höbel S, *et al*. **J Gene Med**. 2010	Mice	Tumor	-F,-OMe,-PS	1.3 μg	Day 0
Jin Hou, *et al*. **Cancer Cell**. 2011	Mice		-Chol,-OMe,-PS	10nmol	Twice/week, 4 weeks
M. DiFiglia, *et al*. **PNAS**. 2007	Mice	Brain	-Chol,-OMe,-PS	0.5 nmol	Day 0
Zhu J, *et al*. **BMC Neurosci**. 2013	Rat		-	0.1nmol	Once every 12 h, 2 days
Kleinman ME, *et al*. **Nature**. 2008	Mice	Eye	-Chol,-OMe	1 μg	Day 0
Soutschek J, *et al*. **Nature**. 2004	Mice	Liver	-Chol,-OMe,-PS	1.25 mg	Continuous 3 days
Tompkins SM, *et al*. **PNAS**. 2004	Mice	Breath	None	20 μg	Day 0
Yuan H, *et al*. **Am J Physiol Renal Physiol**. 2008	Mice	Kidney	-Chol,-OMe,-PS	400 μg	Continuous 7 weeks
Gonzalez-Gonzalez E, *et al*. **Gene Ther**. 2009	Mice	Skin	None	60 μg	Continuous 12 days
Yichao Wu, *et al*. **Cell Host Microbe**. 2009	Mice		-Chol, -PS	2 nmol	Day 0

Related products

Table 4 Related products

Pruduct No.	Product name
C10511-05	ribo*FECT*TM CP Transfection Kit(166T)
C10511-1	ribo*FECT*TM CP Transfection Kit(333T)
C10410-1	ribo*MONITOR*TM Transfection Indicator Kit(Green),20T

Q&A

1. How to set up the experimental control?

A formal experiment includes the experimental group and the negative control group (a-b).Data from experimental group is compared with NC group to determine the inhibitory effect.

In a preliminary experiment, the control group (c-d) was set to confirm that the transfection reagent and the negative control siRNA have no toxic or side effects and no significant interference on the gene silencing indicator.

If the cell transfection efficiency is not well understood, the control group (d) needs to be set up in the preliminary experiment to evaluate the transfection efficiency, so as to determine the appropriate transfection reagent and transfection conditions.

A List of commonly used control groups:

a) Experimental group: Transfection is performed using the siRNA for the gene of interest.

b) Negative control group (NC group): Transfection is performed using a negative control siRNA to demonstrate the specificity of experimental siRNA and as a reference for analyzing the effect of siRNA silencing.

c) Normal cell control group (Blank group): cells without any transfection treatment are used to observe the growth state of the cells throughout the experimental process and as the reference for analysis.

d) Transfection reagent control group (Mock group): transfection with transfection reagent, but no siRNA to exclude the possible influence of transfection reagent on cells.

e) Fluorescently labeled transfection control: Transfection with fluorescent control siRNA or transfection indicator for detection of transfection efficiency of cells under current conditions.

f) Positive control group: Transfection with known effective siRNA to determine the silencing efficiency. High inhibition rate indicates high transfection rate and suitable detection method.

2. Gene silencing is unsatisfactory, how to improve?

1) Improve transfection efficiency

Successful transfection is the prerequisite for RNAi experiments. If the gene silencing effect is not good, please check the transfection efficiency first. The RNAi experimental system can be verified by detecting the transfection efficiency or using the positive control siRNA. First, optimize the transfection conditions (cell density, transfection reagent dosage, transfection time, etc.) according to the transfection reagent instructions. Second, try other cell types for the transfection. If other cells cannot be used due to experimental limitations, consider replacing the transfection reagent or transfection method (eg, electroporation).

2) Detect the target gene expression levels

If the expression level of the gene of interest is low in experimental cells, it is difficult to obtain effective silencing. Generally, the ΔCt value between the target gene and GAPDH or ACTB should be 10 or less, which is suitable for RNAi. If the ΔCt value is 15 or more, it is

hard to achieve an effective silencing. For the case where the expression level of the gene of interest is low, it is recommended to replace the experimental cells.

3) Optimize siRNA concentration

siRNA achieves the desired effect under a certain abundance. Therefore, it is recommended to carry out a pre-experiment under the siRNA concentration recommended by RiboBio (50nmol). Take the data obtained, the experimental cell type and the expression level of the target gene into account to set up the siRNA concentration gradient so as to optimize siRNA concentration.

4) Replace the siRNA

If the transfection effect is good, other factors have been analyzed without problems, but the ideal gene silencing effect is not achieved, you can try to replace the siRNAs. Due to the sequence characteristics of RNA itself or the complex secondary structure of the targeted site, the gene silencing effect of some siRNAs may not be significant. In this case, try to change the siRNA.

5) The detection and analysis method is incorrect

The primers are incorrectly used, the detection time point is unreasonable, the abnormality of the control sample, and the inconsistent test data of the experimental sample will affect the data analysis. Such problems need to be excluded specifically.

6) Other reasons

For example, the expression regulation principals of the target gene make it difficult to be silenced. If the transfection methods, transfection efficiency, gene expression level and siRNA concentration have been checked, and more than 5 pairs of siRNAs have been tried, consider replacing the cells.