User Guide for Pre-designed siRNA Set

Mechanism of siRNA

siRNA (small interfering RNA) is synthesized through chemical methods to obtain two single-stranded RNAs, which then anneal to form double-stranded RNA. The RNA duplex consists of a passenger strand (sense strand) and a guide strand (antisense strand). siRNA can be transfected into cells using transfection reagents or other targeted delivery methods. After entering the cytoplasm, the sense strand is degraded, and the antisense strand binds to the RNA-induced silencing complex (RISC). The antisense strand complementary pairs with homologous target mRNA sequences in the cell, guiding RISC to cleave the target mRNA, leading to mRNA degradation, which further inhibits protein expression.

Storage and Use of siRNA

- **Purification Method**: HPLC
- **Storage Temperature**: -20℃
- **Form**: Solid powder
- **Solvent**: DEPC water (RNase-free water)
- **Usage Instructions**: Centrifuge the tube at 10,000 r/min for 30 seconds, and make sure the powder is concentrated at the bottom of the tube before opening the lid. Dissolve the siRNA and dissolve it to the required storage concentration. It is recommended to aliquot and store the liquid RNA at -20℃, avoided from repeated freeze-thaw cycles.
- **Dilution Method**: 1 OD siRNA ≈ 2.5 nmol. Add 150 μL DEPC water to dissolve 1 OD siRNA to a 20 μM storage concentration. This concentration is for reference only and the required concentration can be prepared as needed.

Contents of the pre-designed siRNA set

Item	Pre-designed siRNA Set A		Pre-designed siRNA Set A	
	Size	# of tube	Size	# of tube
siRNA of the target gene	3×5 nmol	2.5 nmol/tube 6 tubes in total	4×10 nmol	2.5 nmol/tube 16 tubes in total
GAPDH siRNA (Positive control)	2.5 nmol	1 tube	2.5 nmol	1 tube
Negative Control	2.5 nmol	1 tube	2.5 nmol	1 tube
FAM-labeled NC	2.5 nmol	1 tube	2.5 nmol	1 tube

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Guarantee Policy：

When the transfection efficiency reaches >80%, we guarantee that at least one of the three pairs of siRNA designed by us will have a knockdown efficiency >70%. Otherwise, we will redesign and synthesize 2 pairs of siRNA for free. If the re-synthesized siRNA still cannot achieve the guaranteed interference effect, we will provide a refund. Customers need to provide bright/dark field FAM-NC pictures and qPCR raw data to prove that the interference effect does not meet the standard.

Note: This siRNA set only guarantees that the knockdown efficiency at the RNA level reaches more than 70%, and does not guarantee the results at the protein level. If only Western Blot results are provided to prove that the knockdown is ineffective, we will only provide one free redesign and synthesis, and refunds are not applicable.

siRNA Transfection Procedure

The following protocol uses a 24-well plate as an example for experimental operation. All reagent amounts and volumes are calculated per well.

Adherent Cells: One day before transfection, seed 0.5-2.0×10^5 cells per well in 500 μL of medium containing antibiotics. Please note, serum-containing medium can be used at this step. Recommended cell density for transfection is 60%-80%.

1. Preparation of Transfection Complex
   a) Place the transfection reagent at room temperature and mix gently before use.
   b) Transfection reagent-medium mixture preparation: Pipetting 50 μL serum-free medium or OPTI-MEM into a sterile tube, add 2 μL transfection reagent, mix them gently with a pipette, and then incubate the tube at room temperature for 5 minutes.
   c) siRNA-medium mixture preparation: Pipetting 50 μL serum-free medium or OPTI-MEM into another sterile tube, add 2 μL siRNA, mix them gently with a pipette, and then incubate the tube at room temperature for 5 minutes.
   d) Pipetting the transfection reagent-medium mixture to the siRNA-medium mixture, mix them gently with a pipette, incubate the tube at room temperature for 15-20 minutes, and then transfect into the cells immediately.

   Note: 20μM is the recommended storage concentration for siRNA, and 2μl contains 40pmol siRNA.

2. Add 100 μL of the transfection complex to each well of cells, and shake the plate gently to mix thoroughly.

3. After 6-8 hours of transfection, change to complete medium.

4. Incubate at 37℃ for 24-72 hours to detect mRNA expression, and 48-96 hours to detect the protein expression.

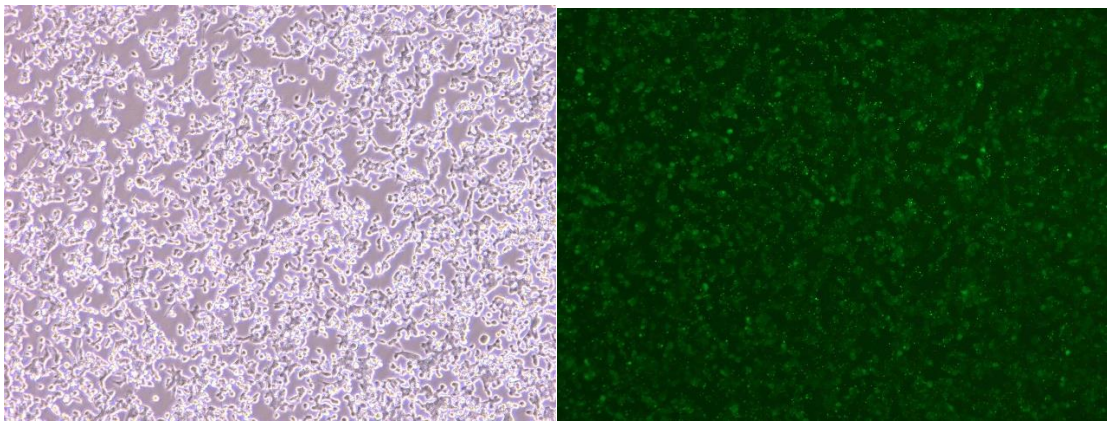
Recommended amount of transfection reagent

Plate Type	Well Area	Medium Amount	Dilution Volume with Opti-MEM	siRNA transfection	
				siRNA amount	Transfection reagent amount
96-well plate	0.3cm^2	100μL	2×10μL	20 pmol	1μl
24-well plate	2.0cm^2	500μL	2×50μL	40 pmol	2μl
12-well plate	4.0cm^2	1mL	2×100μL	80 pmol	4μl
6-well plate	10.0cm^2	2mL	2×200μL	150 pmol	7.5μl
60 mm plate	20.0cm^2	5mL	2×0.5mL	300 pmol	15μl
10 cm plate	60.0cm^2	15mL	2×1mL	600 pmol	30 μl

Experimental Results

1. Transfection Result (HEK293T, 8h post-transfection)

Comparing bright and dark fields, if the proportion of fluorescent cells is over 80% under normal cell growth conditions, the transfection efficiency is >80%.



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2. **qPCR Data & Analysis** (eg. human colon cancer cell line Caco-2, RNF32 gene, 24 hours after transfection)

Group	siRNA name	Target gene	a	Δa	b	c	d	c-d	$2^{-\Delta\Delta Ct}$	$2^{-\Delta\Delta Ct}$ mean	Knockdown efficiency
Negative control	si-NC	RNF32	19.01	18.99	24.22	5.23	5.29	(0.06)	1.04	1.00	0.00%
Negative control	si-NC	RNF32	19.09		24.46	5.47		0.18	0.88		
Negative control	si-NC	RNF32	18.87		24.17	5.18		(0.11)	1.08		
Mock group	None	RNF32	18.49	18.84	24.08	5.24		(0.05)	1.03	1.08	-7.62%
Mock group	None	RNF32	18.98		23.72	4.88		(0.41)	1.33		
Mock group	None	RNF32	19.04		24.33	5.49		0.20	0.87		
Group 1	si-RNF32-1	RNF32	18.88	19.15	24.68	5.53		0.24	0.85	0.84	15.51%
Group 1	si-RNF32-1	RNF32	19.02		24.98	5.83		0.54	0.69		
Group 1	si-RNF32-1	RNF32	19.56		24.45	5.3		0.01	1.00		
Group 2	si-RNF32-2	RNF32	17.56	17.81	23.73	5.92		0.63	0.64	0.59	41.25%
Group 2	si-RNF32-2	RNF32	17.83		23.68	5.87		0.58	0.67		
Group 2	si-RNF32-2	RNF32	18.03		24.25	6.44		1.15	0.45		
Group 3	si-RNF32-3	RNF32	19	18.67	26.47	7.8		2.51	0.18	0.21	78.79%
Group 3	si-RNF32-3	RNF32	18.23		26.03	7.36		2.07	0.24		
Group 3	si-RNF32-3	RNF32	18.79		26.14	7.47		2.18	0.22		
Negative control	Si-NC	GAPDH	19.01	18.99	17.82	-1.17	(1.30)	0.13	0.91	1.00	0.00%
Negative control	Si-NC	GAPDH	19.09		17.32	-1.67		(0.37)	1.29		
Negative control	Si-NC	GAPDH	18.87		17.93	-1.06		0.24	0.85		
Positive control	si-GAPDH	GAPDH	17.68	17.51	23.24	5.73		7.03	0.01	0.01	99.36%
Positive control	si-GAPDH	GAPDH	17.99		23.74	6.23		7.53	0.01		
Positive control	si-GAPDH	GAPDH	16.87		23.59	6.08		7.38	0.01		

Notes:

a = Ct value of the reference gene (beta-actin)

Δa = Mean Ct value of the reference gene for each group

b = Ct value of the target gene

C = ΔCt = Ct value of the target gene - Mean Ct value of the reference gene

d = Mean ΔCt value of the target gene in the NC group

c-d = ΔΔCt = ΔCt value of the target gene - Mean ΔCt value of the reference gene

Knockdown efficiency = (1 - $2^{-\Delta\Delta Ct}$ mean) * 100%

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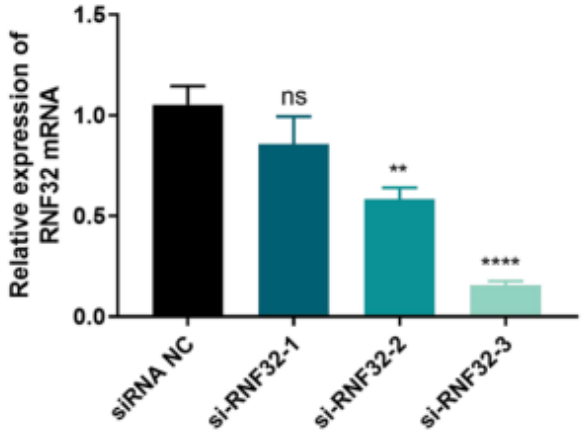
qPCR data analysis:

1) No knockdown effect showed in the NC group.
2) The difference in knockdown efficiency between the Mock group and the NC group is within the range of ±20%, which means there is no actual knockdown efficiency. If it exceeds this range, the Mock group or the NC group needs to find the reason. The Mock group in the above table is increased by 7.62%, which can be considered as no knockdown efficiency, and the experimental results are available.
3) The knockout efficiency of the positive control group should be above 70%. In this experiment, the knockout efficiency of the positive group reached 99.36%.
4) If the knockdown efficiency of any of the Groups 1, 2, and 3 is above 70%, gene silencing is considered to have been achieved. The knockdown efficiency of Group 3 in the above table is >70%, and the siRNA set we designed and delivered has met our guarantee for customers.

Histogram of siRNA interference effect:



3. **After interference with the siRNA set, the results of protein expression are as follows (e.g. human colon cancer cell Caco-2, RNF32 gene, 48 hours after transfection)**