Contents and storage

Refer to the insert in your siRNA library package for content details.

- Store at or below –20°C. Do not store in a frost-free freezer.
- Dried oligonucleotides are shipped at room temperature.
- 12-month shelf life

Product description

- *Silencer*™ Select siRNAs are optimized with the latest design algorithms, proprietary chemical modification and high quality synthesis to ensure desired RNAi outcomes (see best in class information here).
- *Silencer*™ Select libraries include Pre-designed and Validated siRNAs. A few siRNAs target more than one gene's transcript(s), due to gene families with highly homologous members or predicted genes with high homology to verified genes.

Required materials

- RNase-free reagents
- Transfection reagent e.g. Lipofectamine™ RNAiMAX Transfection Reagent

Online resources

- See related products at thermofisher.com/sirna.
- For support, visit thermofisher.com/support.

siRNA resuspension guidelines

We recommend preparing a 5 µM siRNA stock solution.

1. Briefly centrifuge the plate to ensure that the dried siRNA is at the bottom of the wells.
2. Resuspend the siRNA in each well in nuclease-free water at a final concentration of 5 µM (50 µL per 0.25 nmol siRNA).
3. *(Optional)* Aliquot siRNAs into one or more daughter plates to limit the number of freeze-thaw cycles to which the siRNAs are subjected. Solutions at concentrations >2 µM can undergo up to 50 freeze-thaw cycles without significant degradation.
4. Store at or below –20°C in a non-frost-free freezer until use.

Once reconstituted in nuclease-free water, the siRNA is ready to transfect at your choice of final concentration.

- RNA oligonucleotides are susceptible to degradation by exogenous ribonucleases introduced during handling. Wear gloves when handling this product. Use RNase-free reagents, tubes, and barrier pipette tips.

Transfection guidelines

See page 2 to view guidelines for transfection of siRNAs using Lipofectamine™ RNAiMAX Reagent. A range of siRNA concentration and transfection conditions should be optimized prior to siRNA screening for optimal RNA knockdown. We recommend using 25 nM final siRNA concentration as a starting point.

Reverse transfection is faster to perform than forward transfection, and it is the method of choice for high-throughput transfection. Perform reverse transfection by preparing the siRNA transfection complexes inside the wells, and then adding cells and medium. Because the cells and siRNA-reagent complexes are prepared on the same day, we recommend using 2.5× more cells than for forward transfection.

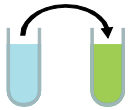
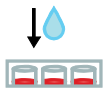
Transfection efficiency varies according to the cell type and transfection agent used. To optimize, determine the conditions that result in maximum gene silencing with minimal cytotoxicity. Maintain conditions across experiments, and use positive and negative controls in all plates

Long non-coding RNA (lncRNA) gene expressions are lower and highly variable across cell types when compared to a protein coding gene. Therefore it is very important to accurately analyze expression levels of your lncRNA prior to performing knockdown experiments especially when using qPCR for measuring % knockdown efficiencies post siRNA transfections.

Limited product warranty and licensing information

Silencer™ Select Human Long Non-Coding RNA (lncRNA) siRNA Library: Transfection Protocol

This procedure is designed for siRNA in combination with Lipofectamine™ RNAiMAX.
IMPORTANT! The prepared mix is sufficient for triplicate transfections with overage.

Step				Action			
Day 0	1		Seed cells to be 60–80% confluent at transfection	Component	384-well	96-well	24-well
				Cell density	0.8–2 × 10^3 cells	0.5–4 × 10^4 cells	0.5–2 × 10^5 cells
Day 1	2	 Tube 1	Dilute Lipofectamine™ RNAiMAX Reagent in Opti-MEM™ Medium	Component (Tube 1)	384-well	96-well	24-well
				Opti-MEM™ Medium	20 µL	25 µL	125 µL
				Lipofectamine™ RNAiMAX Reagent	0.3 µL	1.5 µL	7.5 µL
	3	 Tube 2	Dilute siRNA in Opti-MEM™ Medium	Component (Tube 2)	384-well	96-well	24-well
				Opti-MEM™ Medium	20 µL	25 µL	125 µL
				siRNA (5 µM stock)	1.25 µL (6.25 pmol)	2.5 µL (12.5 pmol)	12.5 µL (62.5 pmol)
	4	 Tube 1 Tube 2	Add diluted siRNA to diluted Lipofectamine™ RNAiMAX Reagent (1:1 ratio) Pipet up and down 5 times to mix. Do not vortex.	Component	384-well	96-well	24-well
				Diluted siRNA	20 µL	25 µL	125 µL
				Diluted Lipofectamine™ RNAiMAX Reagent	20 µL	25 µL	125 µL
	5		Incubate	Incubate complex for 5 minutes at room temperature.			
	6		Add siRNA-lipid complex to cells	siRNA lipid complex	8 µL	10 µL	50 µL
				Final component (per well)	384-well	96-well	24-well
				siRNA (25 nM final)	1.25 pmol	2.5 pmol	12.5 pmol
				Lipofectamine™ RNAiMAX	0.06 µL	0.3 µL	1.5 µL
				Total volume (includes culture medium)	50 µL	100 µL	500 µL
Day 2–4	7		Visualize/analyze transfected cells	Incubate cells for 1–3 days at 37°C, then analyze transfected cells. For details on optimization, see "Transfection guidelines"			