Package contents	Refer to the insert in your siRNA library package for content details.
Storage conditions	▪ 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
Required materials	▪ RNase-free reagents ▪ Transfection reagent e.g. Lipofectamine™ RNAiMAX
Timing	Transfection preparation: 15 minutes Final incubation: 1–3 days
Selection guide	**siRNAs** Go online to view related products.
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.
Handling instructions	▪ 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.
Online resources	Visit the **product page** for additional information and protocols. For support, visit **www.thermofisher.com/support**.

siRNA resuspension guidelines

We recommend preparing 10 µM siRNA stock solutions.

1. Briefly centrifuge the plate to ensure that the dried siRNA is at the bottom of the wells.
2. Resuspend the siRNA in nuclease-free water at a final concentration of 10 µM (25 µL for 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.

Transfection guidelines

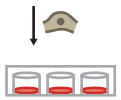
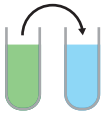
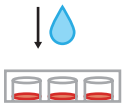
See page 2 to view guidelines for transfection of siRNAs using Lipofectamine™ RNAiMAX Reagent. A range of siRNA concentration and transfection conditions should be optimized prior to siRNA screening for optimal RNA knockdown. We recommend using 10 nM final siRNA concentration as a starting point.

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

Transfection procedure

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

Timeline		Steps	Procedure Details			
			Component	384-well	96-well	24-well
Day 0	1	 **Seed cells to be 60-80% confluent at transfection**	Adherent cells	0.8–2 × 10^3	1–4 × 10^4	0.5–2 × 10^5
Day 1	2	 **Dilute Lipofectamine™ RNAiMAX Reagent in Opti-MEM™ Medium**	Opti-MEM™ Medium	20 µL	25 µL	50 µL
			Lipofectamine™ RNAiMAX Reagent	0.3 µL	1.5 µL	3 µL
	3	 **Dilute siRNA in Opti-MEM™ Medium**	Opti-MEM™ Medium	20 µL	25 µL	50 µL
			siRNA (10 µM stock)	0.25 µL (2.5 pmol)	0.5 µL (5 pmol)	1 µL (10 pmol)
	4	 **Add diluted siRNA to diluted Lipofectamine™ RNAiMAX Reagent (1:1 ratio)**	Diluted siRNA	20 µL	25 µL	50 µL
			Diluted Lipofectamine™ RNAiMAX Reagent	20 µL	25 µL	50 µL
	5	 **Incubate**	Incubate 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 (10 nM final)	0.5 pmol	1 pmol	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.			