Silencer[™] Select Libraries: Transfection Protocol

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	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.			
<u>C</u>	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 $10 \,\mu\text{M}$ (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.

invitrogen

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

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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					
Day 0			Seed cells to be 60-80% confluent at transfection	Component	384-well	96-well	24-well	
	1			Adherent cells	$0.8-2 \times 10^{3}$	$1-4 \times 10^4$	$0.5-2 \times 10^{5}$	
Day 1			Dilute Lipofectamine™ RNAiMAX Reagent in Opti-MEM™ Medium	Opti-MEM [™] Medium	20 µL	25 µL	50 µL	
	2			Lipofectamine [™] RNAiMAX Reagent	0.3 µL	1.5 µL	3 µL	
		\$	Dilute siRNA in Opti-MEM [™] Medium	Opti-MEM [™] Medium	20 µL	25 µL	50 µL	
	3			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	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.				

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