Silencer[®] Select Human Kinase siRNA Library V4

$\mathbf{\mathbf{\mathbf{\mathbf{\mathbf{\mathbf{S}}}}}}$	Package Contents	Catalog NumberSize43979180.25 nmol each siRNA					
	Storage Conditions	 1.75 mL Nuclease-free Water 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					
Å	Selection Guide	siRNAs Go online to view related products.					
<u>C</u>	Product Description	 Silencer[®] Select siRNAs are chemically modified, 21-mer, double-stranded RNAs (dsRNAs) with thir generation locked nucleic acid (LNA) chemistry for increased potency and specificity as compared to unmodified 21-mer dsRNAs (Silencer[®] siRNA). This library targets 710 human kinases with three individual Silencer[®] Select siRNAs per gene. This siRNA library enables systematic, yet cost effective, RNAi studies of these key cell regulators. It is provided in 96-well plates. 					
	Transfection Guidelines	 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. 					
		 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. 					
	Online Resources	Visit our product page for additional information and protocols. For support,					

Library Contents and Target Information

This library contains 2130 unique siRNAs targeting each of 710 human kinase genes*. Contents include a total of 27, 96-well plates (plates are Axygen Catalog No. PCR96FS; www.axygen.com).

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- 24 plates with 88 siRNAs each
- 3 plates with 6 siRNAs each

*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.

siRNA Resuspension Protocol

We recommend preparing 10 µM siRNA stock solution.

- 1. Briefly centrifuge the plate to ensure that the dried siRNA is at the bottom of the tube.
- 2. Resuspend the 0.25 nmol siRNA using 25 μL of the nuclease-free water provided for a final concentration of 10 $\mu M.$
- 3. (Optional) Aliquot siRNAs into one or more daughter tubes or plates to limit the number of freeze-thaw cycles to which the siRNAs are subjected. Solutions at concentrations $>2 \mu M$ 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 and can be used at your choice of final concentration.

RNAi Transfection Protocol

See page 2 to view guidelines for transfecting siRNAs using Lipofectamine[®] RNAiMAX Reagent. We recommend using 10 nM siRNA concentration as a starting point.

Reverse Transfection of RNAi

Reverse transfection is faster to perform than forward transfection and 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 recommended using 2.5× more cells than for a regular transfection.

Limited Product Warranty and Disclaimer Details



For Research Use Only. Not for use in diagnostic procedures.

visit www.lifetechnologies.com/support.

RNAi Transfection Protocol

This procedure is designed for one RNA amount combined with one amount of Lipofectamine[®] RNAiMAX. The prepared mix is enough to have triplicates (96-well), duplicates (24-well), and single well (6-well) transfections, and account for pipetting variations.

	Timeline		Steps	Procedure Details				
Day 0			Seed cells to be	Component	96-well	24-well	6-well	
	1		60-80% confluent at transfection	Adherent cells	$1-4 \times 10^{4}$	$0.5-2 \times 10^{5}$	0.25–1 × 10 ⁶	
Day 1	2		Dilute Lipofectamine [®] RNAiMAX Reagent in Opti-MEM [®] Medium	Opti-MEM [®] Medium	25 μL	50 µL	150 µL	
				Lipofectamine [®] RNAiMAX Reagent	1.5 µL	3 µL	9 µL	
		2	Dilute siRNA in Opti-MEM [®] Medium	Opti-MEM [®] Medium	25 μL	50 μL	150 μL	
	3			siRNA (10 µM)	0.5 μL (5 pmol)	1 μL (10 pmol)	3 μL (30 pmol)	
	4		Add diluted siRNA to diluted Lipofectamine [®] RNAiMAX Reagent (1:1 ratio)	Diluted siRNA	25 μL	50 µL	150 µL	
				Diluted Lipofectamine [®] RNAiMAX Reagent	25 μL	50 µL	150 µL	
	5	5	Incubate	Incubate for 5 minutes at room temperature.				
				Component	96-well	24-well	6-well	
	6	1	Add siRNA-lipid complex to cells	siRNA-lipid complex per well	10 µL	50 µL	250 μL	
				Final siRNA used per well	1 pmol	5 pmol	25 pmol	
				Final Lipofectamine [®] RNAiMAX used per well	0.3 µL	1.5 µL	7.5 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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