








Silencer[®] Select Human Druggable Genome siRNA Extension Set V4, 384-well plates

 Package Contents	Catalog Number 4397924 Size 0.25 nmol each siRNA <ul style="list-style-type: none"> 1.75 mL Nuclease-free Water
 Storage Conditions	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> Silencer[®] Select siRNAs are chemically modified, 21-mer, double-stranded RNAs (dsRNAs) with third generation locked nucleic acid (LNA) chemistry for increased potency and specificity as compared to unmodified 21-mer dsRNAs (Silencer siRNA). This library contains 4,149 unique siRNAs (0.25 nmol) targeting transcripts from each of 1,383 human genes, supplied in 384-well plates.
 Transfection Guidelines	<ul style="list-style-type: none"> 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, visit www.lifetechnologies.com/support.



Library Contents and Target Information

This library contains 4,149 unique siRNAs targeting transcripts from each of 1,383 human genes*. Contents include a total of 12, 384-well plates (plates are Axygen Catalog No. PCR96FS; www.axxygen.com).

- 9 plates with 352 siRNAs each
- 3 plates with 327 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.

- Briefly centrifuge the plate to ensure that the dried siRNA is at the bottom of the tube.
- Resuspend the 0.25 nmol siRNA using 25 μL of the nuclease-free water provided for a final concentration of 10 μM .
- (Optional) Aliquot siRNAs into daughter tubes or plates to limit the number of freeze-thaw cycles to which the siRNAs are subjected. Solutions at concentrations $>2 \mu\text{M}$ can undergo up to 50 freeze-thaw cycles without significant degradation.
- 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 \times more cells than for a regular transfection.

 **Limited Product Warranty and Disclaimer Details**

 **Limited Use Label Licenses**

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	1		Seed cells to be 60-80% confluent at transfection	Component	96-well	24-well	6-well
				Adherent cells	1–4 × 10 ⁴	0.5–2 × 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
	3		Dilute siRNA in Opti-MEM® Medium	Lipofectamine® RNAiMAX Reagent	1.5 µL	3 µL	9 µL
	4		Add diluted siRNA to diluted Lipofectamine® RNAiMAX Reagent (1:1 ratio)	Opti-MEM® Medium	25 µL	50 µL	150 µL
	5		Incubate	siRNA (10 µM)	0.5 µL (5 pmol)	1 µL (10 pmol)	3 µL (30 pmol)
Day 2-4	6		Add siRNA-lipid complex to cells	Diluted siRNA	25 µL	50 µL	150 µL
	7		Visualize/analyze transfected cells	Diluted Lipofectamine® RNAiMAX Reagent	25 µL	50 µL	150 µL
				Incubate for 5 minutes at room temperature.			
				Component	96-well	24-well	6-well
				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
				Incubate cells for 1–3 days at 37°C. Then, analyze transfected cells.			