
Silencer[®] Select Human Druggable Genome siRNA Library V4, 384-well Plates

Product Information Sheet

Product:	Silencer [®] Select Human Druggable Genome siRNA Library V4, 384-well Plates
Catalog # (P/N):	4397922
Lot #:	AMO02KD7
Amount:	0.25 nmol each siRNA
Content:	<p>27,093 unique siRNAs targeting transcripts from each of 9,031 human genes*</p> <p>Total of 78, 384-well plates (plates are Axygen # P384-120SQ-C-S; www.axygen.com)</p> <p>72 plates with 352 siRNAs each</p> <p>3 plates with 351 siRNAs each</p> <p>3 plates with 232 siRNAs each</p> <p>*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. See the accompanying CD for annotation information and siRNA details.</p> <p>For combined files that include this library, <i>Silencer</i> Select Human Druggable Genome V4 siRNA Extension Set, 384-well Plates (P/N 4397924), or <i>Silencer</i> Select Human Genome V4 siRNA Extension Set, 384-well Plates (P/N 4397923) e-mail us at libraries@ambion.com.</p>
Purity:	Standard
siRNA Format:	Annealed
Appearance:	Powder
Storage:	Store at or below -20°C . Do not store in a frost-free freezer. (Dried oligonucleotides are shipped at ambient temperature.)
Shelf Life:	1 year, when stored at or below -20°C .

USER INFORMATION

Product Description:	<p>This Ambion <i>Silencer</i>[®] Select Human Druggable Genome siRNA Library V4 is a 384-well format collection of <i>Silencer</i> Select Pre-designed and, where available, Validated siRNAs targeting RefSeq transcripts from 9,032 human genes that have known or predicted functions similar to previously identified potential therapeutic targets, more commonly known as “druggable” genes. The <i>Silencer</i> Select Human Druggable Genome V4 siRNA Extension Set, 384-well Plates (P/N 4397924) targets transcripts from an additional 1,383 therapeutically relevant genes.</p> <p>The 384-well <i>Silencer</i> Select Human Druggable Genome siRNA Library V4, <i>Silencer</i> Select Human Druggable Genome V4 siRNA Extension Set, and the <i>Silencer</i> Select Human Genome V4 siRNA Extension Set, 384-well Plates (P/N 4397923) comprise the <i>Silencer</i> Select Human Genome siRNA Library V4, 384-well Plates (P/N 4397926), which targets a genome-scale collection of human genes.</p> <p><i>Silencer</i> Select siRNAs are designed using a novel algorithm that was developed utilizing the latest in machine-learning methods. These next-generation siRNAs exhibit up to 100-fold higher silencing potency than siRNAs from other leading siRNA manufacturers. Off-target activity (assayed by microarray analysis) is blocked by up to 90% because <i>Silencer</i> Select siRNAs can be used at 5- to 20-fold lower concentrations, are bioinformatically screened using the latest knowledge about miRNA seed regions and toxic sequence motifs, and incorporate strategic chemical modifications. As a result, <i>Silencer</i> Select siRNAs provide unrivalled specificity and cleaner, more consistent phenotypic data.</p>
Handling Instructions:	<p>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. Upon receipt, your siRNAs may be safely stored in a non-frost-free freezer at or below -20°C (dried oligonucleotides are shipped at ambient temperature).</p>

Resuspension of siRNAs

Centrifuge each plate at low speed (maximum RCF 4,000 X g) to collect the contents at the bottom of the wells before removing the seal.

Important: Perform this process in a tissue culture hood.

1. Remove seal carefully.
2. Add nuclease-free, sterile water, using a multichannel pipette and sterile tips, to achieve the desired concentration.*
3. Gently pipet up and down 5 times to resuspend.
4. Centrifuge briefly to collect the liquid at the bottom of the wells, if necessary.
5. (Optional) Aliquot the siRNAs into one or more daughter plates, to limit the number of freeze-thaw cycles to which the siRNAs are subjected.
6. Place a new sterile seal (such as Axygen PCR-AS-200) on the plate before storing.
7. Store at -80°C until ready to use.

* An online calculator for suspension of dried oligonucleotides is available at www.ambion.com/techlib/append/oligo_dilution.html

Applications:**Transfecting Silencer Select siRNAs Into Mammalian Cells**

The efficiency with which mammalian cells are transfected with siRNA will vary according to cell type and the transfection agent used. Determine the optimal transfection conditions that maximize gene silencing while minimizing cytotoxicity.

Once the conditions for maximal gene silencing are determined, they should be kept constant from experiment to experiment for a given cell type. Include controls in all plates for each experiment to ensure consistency.

Transfection Optimization

Use your experimental cell line, and appropriate positive and negative control siRNAs.

First identify an effective transfection agent for your cell type. Then adjust, in order of importance:

- Amount of transfection agent.
- Amount of siRNA.
- Cell density at the time of transfection. In general, 30–70% confluency is recommended.
- Order of transfection (traditional or reverse).
- Length of exposure of cells to transfection agent/siRNA complexes.

Many protocols recommend maintaining mammalian cells in the medium used for transfection for 48 hours. For many Silencer Select siRNAs, maximal activity is achieved after 24 hours, and the existing medium can be replaced with fresh medium 24 hours after transfection, resulting in greater viability of the cells.

General Transfection Starting Points

When using Silencer Select siRNAs, we suggest starting concentrations of 5- to 20-fold less than typically used for transfection of your experimental cell lines with other siRNAs. We have found that Silencer Select siRNAs at final concentrations of 2 to 10 nM reduced mRNA levels >80%, using lipid-mediated transfection in HeLa and U-2 OS human osteosarcoma cells. To increase accuracy and reproducibility when preparing transfection complexes, prepare a dilution of your stock siRNA, and pipet a higher volume of diluted stock.

General Transfection Starting Points for Mammalian Cells^a

Plate Format	384 wells	96 wells	24 wells	12 wells
Transfection Agent ^b	0.03–0.15 µL	0.2–1.0 µL	1–3 µL	2–4 µL
siRNA ^c	0.25–0.5 pmol	0.5 pmol	2.5 pmol	5 pmol
Cell Density	400–2500 cells/well	6000 cells/well	40,000 cells/well	80,000 cells/well
Final Volume per Well	50–100 µL	100 µL	0.5 mL	1.0 mL

^a Appropriate for lipid-mediated transfection and easily transfected cell lines such as HeLa.

^b Lipofectamine[®] RNAiMAX Transfection Reagent recommended. Refer to the instructions provided with your transfection agent for the recommended volume.

^c The siRNA amounts indicated result in a final siRNA concentration of 5 nM.

For additional information about siRNA transfection, including transfection conditions for many cell types and optimization protocols, see the siRNA Delivery Resource at:

www.ambion.com/techlib/resources/delivery

RELATED PRODUCTS

Silencer[®] Select Pre-designed and Validated siRNAsP/N Various (see www.ambion.com/geneassist)

An all-new class of modified siRNAs with unsurpassed efficacy, potency and specificity. Search the GeneAssist™ Atlas at www.ambion.com/geneassist to find guaranteed-to-silence siRNAs to your gene of interest.

Silencer[®] Select Control siRNAsP/N Various (see www.ambion.com/siRNA)

Validated, nontargeting siRNAs (*Silencer* Select Negative Controls) and proven-to-work positive control siRNAs (*Silencer* Select GAPDH siRNAs) with the *Silencer* Select modifications.

Lipofectamine[®] RNAiMAX Transfection Reagent

PN 13778-150, 13778-075

A proprietary RNAi-specific cationic lipid formulation that offers the highest transfection efficiencies on the widest variety of cell types for siRNA gene knockdown experiments. See www.invitrogen.com.

TaqMan[®] Gene Expression AssaysSee www.allgenes.com or www.ambion.com/geneassist

A comprehensive collection of over 700,000 probe and primer sets for quantitative gene expression analysis using real-time PCR. Search the GeneAssist™ Atlas at www.ambion.com/geneassist to find suggested TaqMan Gene Expression Assays corresponding to your siRNA targets of interest.

TaqMan[®] Gene Expression Cells-to-Ct™ Kit

P/N AM1728, AM1729

A robust set of lysis, reverse transcription, and PCR reagents that enables streamlined real-time RT-PCR analysis of cultured-cell lysates with user-supplied TaqMan[®] Gene Expression Assays.

QUALITY CONTROL

Identity: The mass of a sample of each single-stranded RNA oligonucleotide is analyzed using MALDI-TOF mass spectrometry and compared to the calculated mass.

Annealing: A sample of the annealed siRNA is analyzed by nondenaturing gel electrophoresis.

OTHER INFORMATION

Safety Data Sheets: Safety Data Sheets (SDSs; previously known as MSDSs) for any chemical product supplied by Applied Biosystems or Ambion are available 24 hours a day. At www.appliedbiosystems.com, select **Support**, then **SDS/MSDS**. Search by chemical name, product name, product part number, or SDS/MSDS part number. Right-click to print or download the SDS of interest. At www.ambion.com, go to the web catalog page for the product of interest. Select **SDS/MSDS**, then right-click to print or download. Or, e-mail (MSDS_Inquiry_CCRM@lifetech.com), telephone (650-554-2756; USA), or fax (650-554-2252; USA) your request, specifying the catalog or part number(s) and the name of the product(s). We will e-mail the associated SDSs unless you request fax or postal delivery. Requests for postal delivery require 1-2 weeks for processing.

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