Silencer® Select Human Druggable Genome V4 siRNA Extension Set, 384-well Plates Product Information Sheet

Product:	*Silencer*® Select Human Druggable Genome V4 siRNA Extension Set, 384-well Plates
Catalog # (P/N):	4397924
Lot #:	XXXXXXXX
Amount:	0.25 nmol each siRNA
Content:	4,149 unique siRNAs targeting transcripts from each of 1,383 human genes* Total of 12, 384-well plates (plates are Axygen #PCR96FS; www.axygen.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. See the accompanying CD for annotation information and siRNA details. For combined files that include this library and *Silencer* Select Human Druggable Genome siRNA Library V4, 384-well Plates (P/N 4397922), or *Silencer* Select Human Genome V4 siRNA Extension Set, 384-well Plates (P/N 4397923), e-mail us at libraries@ ambion.com.
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:	This Ambion *Silencer*® Select Human Druggable Genome V4 siRNA Extension Set is a 384-well format collection of *Silencer* Select Pre-designed and, where available, Validated siRNAs targeting RefSeq transcripts from 1,383 therapeutically relevant human genes. This product is intended to build on the *Silencer* Select Human Druggable Genome siRNA Library V4, 384-well Plates (P/N #4397922), a collection of siRNAs targeting 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 384-well *Silencer* Select Human Druggable Genome siRNA Library V4, *Silencer* Select Human Druggable Genome V4 siRNA Extension Set, and Silencer Select Human Genome V4 siRNA Extension Set, 384-well Plates (P/N 4397923) comprise the *Silencer* Select Human Genome siRNA Library V4, 384-well Plates (P/N 4397926), which targets transcripts from a genome-scale collection of human genes. *Silencer* 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 *Silencer* 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, *Silencer* Select siRNAs provide unrivalled specificity and cleaner, more consistent phenotypic data.
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. 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).

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. This means that the optimal concentration used for transfections should be determined empirically. Since *Silencer* Select siRNAs exhibit superior silencing potency compared to other siRNAs, we suggest starting concentrations of 5- to 20-fold less than typically used for transfection of your experimental cell lines. We have found that *Silencer* Select siRNAs reduced mRNA levels >80% at final concentrations of 2–10 nM using lipid-mediated transfection in HeLa and U-2 OS human osteosarcoma cells.

General Transfection Starting Points for Mammalian Cells[a]

Plate Format	384 wells	96 wells	24 wells	12 wells
Transfection Agent[b]	0.3 µL	0.3–1.0 µL	1–3 µL	2–4 µL
siRNA[c]	0.35–0.5 pmol	0.5 pmol	2.5 pmol	5 pmol
Cell Density[d]	4000–8000 cells/well	6000 cells/well	40,000 cells/well	80,000 cells/well
Final Volume per Well	70–100 µL	100 µL	0.5 mL	1.0 mL

[a] Appropriate for lipid-mediated transfection and easily transfected cell lines such as HeLa.
[b] 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. The amount of siRNA required for maximal gene silencing will vary among cell types. For a 96-well plate, and a 100 µL final transfection volume, 0.5 pmol of a 1 µM siRNA solution is 0.5 µL. Robotic pipettors may require volumes of 2–5 µL for accurate pipetting. To increase pipetting volumes and accuracy when preparing transfection complexes, we recommend first preparing a plate with a dilution of your stock siRNA.
[d] Optimal cell density will vary among cell types, depending on cell size and growth characteristics. In general, 30–70% confluency is recommended.

Transfection Optimization

Optimizing transfection efficiency is crucial for maximizing gene silencing while minimizing cytotoxicity. Optimal transfection efficiencies are achieved by identifying an effective transfection agent for each cell type and by adjusting (in order of importance):

- Amount of transfection agent
- Amount of siRNA
- Cell density at the time of transfection
- Order of transfection (pre-plating cells or plating cells/transfecting in tandem)
- Length of exposure of cells to transfection agent/siRNA complexes

Most protocols recommend maintaining mammalian cells in the medium used for transfection; this avoids diluting or removing the siRNAs from the cells by adding medium or washing the cells with new medium too soon after transfection. We have found that cells typically exhibit greater viability when existing medium is replaced by fresh medium 24 hours after transfection. Replacing medium after 24 hours generally does not change the activity of the transfected siRNAs.

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.

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 siRNAs
P/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 siRNAs
P/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.

siPORT™ *NeoFX*™ Transfection Agent
P/N AM4510 and AM4511
A versatile lipid-based agent for efficient and reproducible transfection of adherent cells while subculturing, without increased cytotoxicity.

TaqMan® Gene Expression Assays
See 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 for the gene targeted by an siRNA 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

Material Safety Data Sheets: Material Safety Data Sheets (MSDSs) can be printed or downloaded from product-specific links on our website at the following address: www.ambion.com/techlib/msds. Alternatively, e-mail your request to MSDS_Inquiry_CCRM@appliedbiosystems.com. Specify the catalog or part number(s) of the product(s), and we will e-mail the associated MSDSs unless you specify a preference for fax delivery. For customers without access to the internet or fax, our technical service department can fulfill MSDS requests placed by telephone or postal mail. (Requests for postal delivery require 1–2 weeks for processing.)

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