Silencer® Human Druggable Genome siRNA Library V3.1

Store at or below –20°C.
Do not store in a frost-free freezer.

Catalog # (P/N):	AM80990V3.1
Product Description:	Total of 31132 unique siRNAs targeting each of 7783 genes; 4 siRNAs per gene.
Amount:	0.25 nmol each siRNA
Components:	***Silencer*® Human Druggable Genome siRNA Library V3 (P/N 4384342)** 23,349 unique siRNAs targeting each of 7,783 genes; 3 siRNAs per gene 267 96-well plates* 261 plates with 88 siRNAs each 3 plates with 87 siRNAs each 3 plates with 40 siRNAs each ***Silencer*® Human Druggable Genome siRNA Library V3, 4th siRNA (P/N 4384345)** A 4th unique siRNA targeting each of 7783 genes corresponding to the *Silencer*® Human Druggable Genome siRNA Library V3 89 96-well plates* 87 plates with 88 siRNAs each 1 plate with 87 siRNAs 1 plate with 40 siRNAs Total of 356 plates. Each component includes a CD with sequence information and additional siRNA details for that library subset. For a combined file, e-mail us at libraries@ambion.com. * Plates are Axygen #PCR96FS; www.axygen.com.
Purity:	Standard
Format:	Annealed
Appearance:	Powder
Storage Conditions:	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

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 in step 1.

Important: Perform this process under a tissue culture hood to prevent contamination of siRNA stock.

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. (Optional) Aliquot the siRNAs into one or more daughter plates, to limit the number of freeze-thaw cycles to which the siRNAs are subjected.
5. Place a new sterile seal (such as Axygen Cat #PCR-AS-200) on the plate before storing.
6. Store at –80°C until ready to use.

* Ambion provides an online calculator for resuspension of dried oligonucleotides on its website at www.ambion.com/techlib/append/oligo_dilution.html

Applications:

Transfecting *Silencer* 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.

We have found that *Silencer* siRNAs typically work best when present in cell culture medium at 10–50 nM; however, a more extensive concentration range from 1–100 nM can be analyzed in optimization experiments. To increase accuracy and reproducibility when preparing transfection complexes, prepare a dilution of your stock siRNA, and pipet a higher volume of diluted stock.

Transfection Optimization
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 (in general, 30–70% confluency recommended)
- Order of transfection (traditional or reverse transfection)
- Length of exposure of cells to transfection agent/siRNA complexes

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.

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	3 pmol	15 pmol	30 pmol
Cell Density	400–2,500 cells/well	6,000 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 30 nM.

Many protocols recommend maintaining mammalian cells in the medium used for transfection for 48 hours. For many *Silencer* 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.

For additional information about siRNA transfection, including transfection conditions for many cell types and optimization protocols, see the siRNA Delivery Resource at:
www4.appliedbiosystems.com/techlib/resources/delivery

RELATED PRODUCTS

***Silencer*® Control siRNAs**
P/N Various (see www.ambion.com/siRNA)
Validated, nontargeting siRNAs (negative controls) and siRNAs targeting genes such as GAPDH, β-actin, and GFP (positive controls).

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 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 corresponding to your siRNA targets of interest.

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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For research use only. Not intended for any animal or human therapeutic or diagnostic use.

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