

Silencer® Select Pre-designed siRNA
Silencer® Select Validated siRNA
Silencer® Select Custom Designed siRNA
Custom Select siRNA

General Product Details and User Information

Refer to page 3 for product descriptions.

Format:	Annealed
Appearance:	Powder
Additional Material(s) Included:	1.75 mL Nuclease-free Water (40 nmol size and smaller)
Storage Conditions:	Store at or below -20°C . Do not store in a frost-free freezer. (Dried oligonucleotides are shipped at ambient temperature.)
Safety Information:	Read the Safety Data Sheet, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

USER INFORMATION

Product Description:	Ambion® <i>Silencer</i> ® Select siRNAs are designed using a novel algorithm that was developed utilizing the latest advances 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.
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> <p>Resuspension of siRNA Briefly centrifuge the tube to ensure that the dried siRNA is at the bottom of the tube. Resuspend siRNA at a convenient concentration. For example, resuspend 40 nmol of siRNA in 800 μL of the Nuclease-free Water provided for a final concentration of 50 μM.</p> <p>An online calculator for suspension of dry oligonucleotides is available at www4.appliedbiosystems.com/techlib/append/oligo_dilution.html</p> <p>Once reconstituted in Nuclease-free Water, the siRNA is ready to transfect and can be used at your choice of final concentration.</p> <p>Store the resuspended siRNA at or below -20°C. After resuspension, siRNA stock solutions at concentrations $\geq 2 \mu\text{M}$ can undergo up to 50 freeze-thaw cycles without significant degradation. However, storage in a frost-free freezer is not recommended. For long-term storage, RNA oligonucleotides may be stored at or below -70°C.</p>
Applications:	<p>Transfecting <i>Silencer</i> 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 <i>Silencer</i> 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 <i>Silencer</i> 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.</p>

General Transfection Starting Points for Mammalian Cells ^a

Plate Format	96 wells	24 wells	12 wells	6 wells
Transfection Agent ^b	0.2–1.0 μ L	1–3 μ L	2–4 μ L	3–6 μ L
siRNA ^c	0.5 pmol	2.5 pmol	5 pmol	12.5 pmol
Cell Density ^d	6,000 cells/well	40,000 cells/well	80,000 cells/well	200,000 cells/well
Final Volume per Well	100 μ L	500 μ L	1.0 mL	2.5 mL

a Appropriate for lipid-mediated transfection and easily transfected cells 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 μ L final transfection volume, 0.5 pmol of a 1 μ M siRNA solution is 0.5 μ L. Robotic pipettors may require volumes of 2–5 μ L 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 dilution or removal of 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 with 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:
www4.appliedbiosystems.com/techlib/resources/delivery

RELATED PRODUCTS

Silencer[®] Select Control siRNAs

P/N Various (see www4.appliedbiosystems.com/geneassist)

Validated, nontargeting siRNAs (negative controls) and siRNA targeting GAPDH (positive control), all including Silencer Select strategic chemical modifications.

Lipofectamine[™] RNAiMAX Transfection Reagent

P/N 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

www.allgenes.com or www4.appliedbiosystems.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 www4.appliedbiosystems.com/geneassist to find suggested TaqMan Gene Expression Assays for the gene targeted by an siRNA of interest.

QUALITY CONTROL

A sample of each RNA oligonucleotide is analyzed by MALDI-TOF mass spectrometry. Analytical HPLC is used to monitor purity of HPLC-purified oligonucleotides. Annealing of siRNAs is assessed by gel electrophoresis.

Pre-designed, Validated, and Custom Designed *Silencer*[®] Select siRNAs

Custom Select siRNAs

Silencer[®] Select Pre-designed siRNAs

An all-new class of modified siRNAs with unsurpassed efficacy, potency and specificity

Type	Purification Method	Purity	Amount	Catalog #
Pre-designed	Standard	≥80%	1 nmol	4427037
Pre-designed	Standard	≥80%	5 nmol	4392420
Pre-designed	Standard	≥80%	20 nmol	4392421
Pre-designed	Standard	≥80%	40 nmol	4392422
Pre-designed, non-inventoried	Standard	≥80%	5 nmol	4390771
Pre-designed, non-inventoried	Standard	≥80%	20 nmol	4390815
Pre-designed, non-inventoried	Standard	≥80%	40 nmol	4390816
Pre-designed	HPLC purified	≥95%	20 nmol	4390817
Pre-designed	HPLC purified	≥95%	40 nmol	4390818
Pre-designed	HPLC purified	≥95%	250 nmol	4404014
Pre-designed	HPLC purified	≥95%	1 μmol	4444246
Pre-designed	HPLC purified	≥90%	10 μmol	4444198

Silencer[®] Select Validated siRNAs

Functionally proven and guaranteed to reduce target mRNA levels by ≥80%

Type	Purification Method	Purity	Amount	Catalog #
Validated	Standard	≥80%	1 nmol	4427038
Validated	Standard	≥80%	5 nmol	4390824
Validated	Standard	≥80%	20 nmol	4390825
Validated	Standard	≥80%	40 nmol	4390826
Validated	HPLC purified	≥95%	20 nmol	4390821
Validated	HPLC purified	≥95%	40 nmol	4390822
Validated	HPLC purified	≥95%	250 nmol	4407270
Validated	HPLC purified	≥95%	1 μmol	4444260
Validated	HPLC purified	≥90%	10 μmol	4444247

Custom Select siRNAs

Made-to-order siRNAs with the *Silencer*[®] Select modifications

Type	Purification Method	Purity	Amount	Catalog #
Custom Select	Standard	≥80%	20 nmol	4390828
Custom Select	Standard	≥80%	40 nmol	4390829
Custom Select	HPLC purified	≥95%	20 nmol	4390830
Custom Select	HPLC purified	≥95%	40 nmol	4390831
Custom Select	HPLC purified	≥95%	250 nmol	4404013
Custom Select	HPLC purified	≥95%	1 μmol	4444245
Custom Select	HPLC purified	≥90%	10 μmol	4444207

Silencer® Select Custom Designed siRNAs
Custom siRNA designs for any target, using the *Silencer* Select algorithm

Type	Purification Method	Purity	Amount	Catalog #
Custom Designed	Standard	≥80%	5 nmol	4399665
Custom Designed	Standard	≥80%	20 nmol	4399666
Custom Designed	Standard	≥80%	40 nmol	4399667
Custom Designed	HPLC purified	≥95%	20 nmol	4399668
Custom Designed	HPLC purified	≥95%	40 nmol	4399669

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@appliedbiosystems.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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Silencer® Select Pre-designed Guarantee: Ambion guarantees that when you purchase two *Silencer* Select Pre-designed siRNAs to the same target, both will knock down target mRNA levels in cultured cells at least 70% when measured 48 hours after transfection at 5 nM or higher final siRNA concentration under the conditions described below. If both *Silencer* Select Pre-designed siRNAs do not induce ≥70% target mRNA knockdown, Ambion will provide a one-time replacement of up to two *Silencer* Select Pre-designed siRNAs per target at no additional charge. When you purchase three *Silencer* Select Pre-designed siRNAs to the same target, Ambion guarantees that at least two will knock down target mRNA levels in cultured cells at least 80% when measured 48 hours after transfection at 5 nM or higher final siRNA concentration under the conditions described below. If at least two of the three *Silencer* Select Pre-designed siRNAs do not induce ≥80% target mRNA knockdown, Ambion will provide a one-time replacement of up to two *Silencer* Select Pre-designed siRNAs per target at no additional charge. Requests for replacement product must be made within one hundred and eighty (180) days from the date of delivery of the *Silencer* Select Pre-designed siRNAs.

Optimum transfection efficiency must be confirmed using good laboratory practices and a proven-to-work siRNA to an endogenous message, such as Ambion *Silencer* Select GAPDH siRNA Control. To assess knockdown, target mRNA levels in treated samples must be compared to that of cells transfected with a nontargeting control siRNA, such as *Silencer* Select Negative Control #1. We recommend Applied Biosystems TaqMan® Gene Expression Assays to quantify mRNA levels.

This guarantee does not extend to any replacement product. PRODUCT REPLACEMENT AS SET FORTH ABOVE IS AMBION'S SOLE OBLIGATION AND CUSTOMER'S SOLE AND EXCLUSIVE REMEDY WITH RESPECT TO THE GUARANTEE GIVEN ABOVE.

To arrange a replacement, contact Technical Services. Please be ready to supply data indicating transfection efficiency and measurement of mRNA knockdown.

Silencer® Select Validated siRNA Guarantee: Each *Silencer* Select Validated siRNA is guaranteed to knock down target mRNA levels 80% or more in cultured cells when measured 48 hours after transfection at 5 nM or higher final siRNA concentration under the conditions described below. If a *Silencer* Select Validated siRNA does not induce target knockdown as described above, Ambion will provide a one-time replacement of another *Silencer* Select Validated siRNA, or, if none are available, a *Silencer* Select Pre-designed siRNA to the same target, at no additional charge. Requests for replacement product must be made within one hundred and eighty (180) days from the date of delivery of the *Silencer* Select Validated siRNA.

Optimum transfection efficiency must be confirmed using good laboratory practices and a proven-to-work siRNA to an endogenous message, such as Ambion *Silencer* Select GAPDH siRNA Control. To assess knockdown, target mRNA levels in treated samples must be compared to that of cells transfected with a nontargeting control siRNA, such as *Silencer* Select Negative Control #1. We recommend Applied Biosystems TaqMan® Gene Expression Assays to quantify mRNA levels.

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