Silencer® Select GAPDH siRNA (Hs, Mm, Rn)

Store at or below –20°C.

Catalog #:	4390850	
Amount:	40 nmol	
Appearance:	Powder	
Target Information:	Gene Symbol:	GAPD
	Full Gene Name:	Glyceraldehyde-3-phosphate dehydrogenase
	Organism(s):	Human, Mouse, and Rat
	RefSeq Number(s):	NM_002046 (human), NM_001001303 (mouse), and NM_017008 (rat)
	Entrez Gene ID(s):	2597 (human), 407972 (mouse), and 24383 (rat)
Format:	Annealed	
Additional Material(s) Included:	1.75 mL Nuclease-free Water	
Storage Conditions:	Store at or below –20°C. ***Do not store in a frost-free freezer.*** (Dried oligonucleotides are shipped at ambient temperature.)	

USER INFORMATION

Product Description:

Ambion® *Silencer*® Select siRNAs are designed using an all-new 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.

Silencer® Select GAPDH siRNA (Hs, Mm, Rn) is ideal for developing and optimizing *Silencer* Select siRNA transfection conditions. It can also be used as a control in siRNA experiments to confirm that the transfection procedure and cell cultures support gene silencing. The *Silencer* Select GAPDH siRNA (Hs, Mm, Rn) sequence has been verified to efficiently silence GAPDH in human, mouse, and rat cells, and includes *Silencer* Select modifications. The sense and antisense siRNA strands are chemically synthesized, HPLC purified, and then annealed. The siRNA is shipped in dried form, with Nuclease-free Water provided for resuspension.

Silencer Select GAPDH siRNA (Hs, Mm, Rn) has been successfully used to elicit specific gene silencing in multiple cell lines, including, but not limited to, HeLa, U-2 OS human osteosarcoma cells, and Huh7 human hepatoma cells. GAPDH mRNA levels in transfected and nontransfected cells were measured by real-time RT-PCR using total RNA isolated 48 hr after transfection. *Silencer* Select GAPDH siRNA reduced the expression of GAPDH by 70–95% in every cell line tested.

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 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.

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

Once reconstituted in Nuclease-free Water, the siRNA is ready to transfect and can be used at your choice of final concentration.

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

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	96 wells	24 wells	12 wells	6 wells
Transfection Agent [b]	0.3–1.0 µL	1–3 µL	2–4 µL	3–6 µL
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 µL	500 µL	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 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 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 Ambion's siRNA Delivery Resource at:
www.ambion.com/techlib/resources/delivery

RELATED PRODUCTS

KDalert™ GAPDH Assay Kit
Cat #AM1639
A rapid, sensitive assay system to quantitate siRNA-induced GAPDH knockdown (KD) in cultured cells.

Anti-GAPDH, Mouse Monoclonal 6C5
Cat #AM4300
Ideal for detecting knockdown of GAPDH at the protein level by Western blot or immunofluorescence.

***Silencer*® Select Pre-designed and Validated siRNAs**
Cat #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.

siPORT™ *NeoFX*™ Transfection Agent
Cat #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
www.allgenes.com
A comprehensive collection of over 700,000 probe and primer sets for quantitative gene expression analysis using real-time PCR.

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.

Purity: Analytical HPLC of a sample of the final purified single-stranded RNA oligonucleotides is used to confirm ≥95% purity.

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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