Silencer® GAPDH siRNA (Human, Mouse & Rat)

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

Catalog #:	AM4624	
Component:	GAPDH siRNA	Negative Control #1 siRNA
Amount:	5 nmol	2 nmol
Volume:	100 µL	40 µL
Concentration:	50 µM	50 µM
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	
Purity:	HPLC purified	
Storage Conditions:	Store at or below –20°C. ***Do not store in a frost-free freezer.***	
Storage Buffer:	20 mM potassium acetate, 6 mM HEPES-KOH pH 7.4, 0.4 mM magnesium acetate.	

USER INFORMATION

Product Description:

Ambion *Silencer*® GAPDH siRNA for Human, Mouse, and Rat is ideal for developing and optimizing siRNA transfection conditions in human, mouse, and rat cell lines. It can also be used as a control in siRNA experiments to confirm that the transfection procedure and cell cultures support gene silencing. The sense and antisense siRNA strands are chemically synthesized, HPLC purified, and then annealed. The siRNAs are provided ready to transfect.

The Negative Control siRNA has no significant homology to any known gene sequences from mouse, rat, or human.

GAPDH and Negative Control siRNAs have been successfully used in multiple human, mouse and rat cell lines, including HeLa, 3T3, PC12, and many other cell lines. The GAPDH mRNA level in transfected and nontransfected cells has been measured by real-time RT-PCR using total RNA isolated 48 hr after transfection. 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.

Applications:

Transfecting 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. We have found that 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.

General Transfection Starting Points for Mammalian Cells

Plate Format	96 wells	24 wells	12 wells	6 wells
Transfection Agent [a]	0.3–1.0 µL	1–3 µL	2–4 µL	3–6 µL
siRNA [b]	3 pmol	15 pmol	30 pmol	75 pmol
Cell Density [c]	6,000 cells/well	40,000 cells/well	80,000 cells/well	200,000 cells/well
Final Volume per Well	100 µL	0.5 mL	1.0 mL	2.5 mL

a Refer to the instructions provided with your transfection agent for the recommended volume.

b The siRNA amount shown results in a final siRNA concentration of 30 nM. The amount of siRNA required for maximal gene silencing will vary among cell types. For a 96-well plate and 100 µL final transfection volume, 3 pmol of a 5 µM siRNA solution is 0.6 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 making a plate with a dilution of your stock siRNA.

c Optimal cell density will vary among cell types, depending on cell size and growth characteristics. In general, we recommend 30–70% confluency.

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

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

***Silencer*® Pre-designed and Validated siRNAs**
Cat #Various (see www.ambion.com/siRNA)
Guaranteed-to-silence siRNAs available to all human, mouse, and rat genes. Search the Ambion siRNA database (www.ambion.com/siRNA) to find siRNAs to your genes 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.

siPORT™ *Amine* Transfection Agent
Cat #AM4502 and AM4503
An easy-to-use blend of polyamines that delivers siRNA into mammalian cells with minimal 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.
Suitability for Tissue Culture:	No bacterial growth detected in mammalian tissue culture medium after incubation for 72 h at 37°C in the presence of siRNA.

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.)

Warranty and Liability:

For research use only. Not for use in diagnostic procedures.

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