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Cat #AM4650

Silencer® FAM™ GAPDH siRNA (Human, Mouse, Rat)

Store at or below –70˚C

Description:	A FAM™-labeled double-stranded RNA oligonucleotide designed for monitoring uptake of siRNAs by fluorescence microscopy or other fluorescence-based techniques.
Amount:	5 nmol
Appearance:	Powder
Additional Reagents Supplied:	1.75 mL Nuclease-free Water
Target Information:	Gene symbol: GAPD Full Gene Name: glyceraldehyde-3-phosphate dehydrogenase Organism: Human, Mouse and Rat RefSeq Number(s): NM_002046 (human), NM_001001303 (mouse), and NM_017008 (rat) Entrez Gene ID: 2597 (human), 407972 (mouse) and 24383 (rat)
Spectral Information:	**Dye-conjugated siRNA** 1 OD_{260} = 40 µg/mL **Unconjugated dye** Excitation max (λ_{max}) 494 nm Emission max (λ_{max}) 520 nm ε_{260} [L/(mol cm)] 21,000 L/(mol cm) ε_{494} [L/(mol cm)] 75,000 L/(mol cm)
Storage:	Store at or below –70°C. ***Do not store in a frost-free freezer.*** This product is guaranteed for 1 year from the date of shipment, if properly stored.

QUALITY CONTROL

Identity:	A MALDI-TOF mass spectrometer is used to identify the correct mass of the single-stranded RNA oligonucleotides.
Purity:	Analytical HPLC of purified unlabeled and labeled single-stranded RNA oligonucleotides is used to confirm >95% purity and, where applicable, coupling of dye to nucleic acid.
Annealing:	The annealed siRNA is analyzed by nondenaturing PAGE.

USER INFORMATION

General Information:

Ambion's *Silencer*® FAM™ GAPDH siRNA (Human, Mouse, Rat) has the same sequence as *Silencer*® GAPDH siRNA (Human, Mouse, Rat; Cat #AM4631). It has a fluorescent moiety on the 5' end of one strand and is designed for monitoring delivery in transfection experiments using *Silencer*® siRNAs and human, mouse, or rat cells. This product is shipped in dried form. Nuclease-free water is provided for resuspension.

The fluorescence label enables direct observation of cellular uptake, distribution, and localization of labeled siRNAs. The most common application for dye-labeled siRNAs is to monitor transfection efficiency during optimization of transfection conditions.

Transfect the FAM-labeled GAPDH siRNA using the same methodology as for your experimental siRNAs.

Cells transfected with FAM-labeled GAPDH siRNA can be examined by methods such as fluorescence microscopy, confocal microscopy, or flow cytometry. For observation of FAM-labeled oligonucleotides by fluorescence microscopy, a fluorescein isothiocyanate (FITC) or GFP filter can be used.

General Handling Instructions:

siRNAs are susceptible to degradation by exogenous ribonucleases introduced during handling. As a precaution, your siRNAs should not be handled with ungloved hands. RNase-free reagents, barrier pipette tips, and tubes should be used. Upon receipt, your siRNAs may be safely stored in a non-frost-free freezer at or below –70°C.

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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 5 nmol of siRNA in 100 µL of the Nuclease-free Water provided for a final concentration of 50 µM.

Ambion provides an online calculator for suspension of dry oligonucleotides on its web site at http://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 (e.g., 1–100 nM).

Store the resuspended siRNA at or below –70°C. Do not store in a frost-free freezer.

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:

The following chart provides helpful starting points for transfection of siRNA into cultured mammalian cells.

	96 well	24 well	12 well	6 well
Transfection Agent[a] (µL)	0.3–1.0	1–3	2–4	3–6
siRNA[b] (pmol)	3	15	30	75
Cell Density[c] (cells/well)	6,000	40,000	80,000	200,000
Final Volume per Well (mL)	0.1	0.5	1.0	2.5

[a] Refer to the instructions provided with your transfection agent for the recommended volume.
[b] The siRNA amount shown results in an siRNA concentration of 30 nM. The amount of siRNA required for maximal gene silencing will vary among cell types.
[c] 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 cytoxicity. Optimal transfection efficiencies are achieved by identifying an effective transfection agent for each cell type and by adjusting (by 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. Ambion scientists 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 for a given cell type, they should be kept constant in experiments using that cell type.

For additional information about siRNA transfection, including transfection conditions for many cell types and optimization protocols, see Ambion's siRNA Delivery Resource at:
http://www.ambion.com/techlib/resources/delivery

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RELATED PRODUCTS

***Silencer*® Cy™3 GAPDH siRNA (Human, Mouse, Rat)**
Cat #AM4649
Designed for monitoring uptake of siRNAs by fluorescence-based techniques.

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

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.

***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 Ambion's siRNA database (www.ambion.com/siRNA) to find siRNAs to your genes of interest.

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 or postal mail delivery. For customers without internet access, our technical service department can fulfill MSDS requests placed by telephone or postal mail.

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