# *Silencer*® FAM™-Labeled Negative Control #1 siRNA

**Store at or below –70°C.**

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|---|---|
| **Catalog #:** | AM4620 |
| **Amount:** | 5 nmol |
| **Appearance:** | Powder |
| **Molecular Weight:** | 13837.6 |
| **Spectral Information:** | **Dye-conjugated siRNA**<br>1 $OD_{260}$ = 40 µg/mL<br><br>**Unconjugated dye**<br>Excitation max ($\lambda_{max}$): 494 nm<br>Emission max ($\lambda_{max}$): 520 nm<br>$\varepsilon_{260}$: 21,000 L/(mol·cm)<br>$\varepsilon_{494}$: 75,000 L/(mol·cm) |
| **Format:** | Annealed |
| **Purity:** | HPLC purified |
| **Additional Material(s) Included:** | 1.75 mL Nuclease-free Water |
| **Storage Conditions:** | Store at or below –70°C. ***Do not store in a frost-free freezer.*** |

## USER INFORMATION

**Product Description:**

Ambion *Silencer*® FAM™-Labeled Negative Control #1 siRNA is designed for monitoring uptake of siRNAs by fluorescence microscopy or other fluorescence-based techniques. It has the same sequence as *Silencer*® Negative Control #1 siRNA (Cat #AM4611) and contains a fluorescent moiety on the 5' end of one strand. 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 Negative Control siRNA using the same methodology as for your experimental siRNAs.

Cells transfected with FAM-labeled Negative Control 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.

**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 –70°C.

**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
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 resuspended dye-labeled siRNA at or below –70°C. ***Do not store in a frost-free freezer.***

**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.

In general, siRNAs are rapidly (<4 hr) taken up by cells after transfection and are distributed throughout the cell. Under optimal transfection conditions, cellular uptake of labeled siRNAs is observable at final concentrations in the low nanomolar range, and uptake increases up to 50–100 nM.

After chemical transfection (e.g., lipid-mediated transfection), labeled siRNAs appear to be actively taken up by endosomes. Several hours post-transfection, a dotted perinuclear localization of the labeled siRNA can often be observed.

In contrast, labeled siRNAs delivered to cells by electroporation, a passive uptake process, produce an even, but less intense, glow throughout the cytoplasm. Because of its brighter signal, Cy dyes are recommended over fluorescein or FAM for use in electroporation applications.

Different transfection reagents interact with siRNAs in different ways. Some reagents may associate strongly with the siRNA and sequester it, resulting in poor gene knockdown, despite there being an abundant presence of the siRNA in the cell. Thus, fluorescence of labeled siRNAs in the cell may not always be correlated with knockdown, and an independent experiment should be performed to ascertain if acceptable knockdown of mRNA or protein can be achieved using the transfection reagent and cell line under study. Once this is done, the extent of gene knockdown can be accurately correlated with transfection efficiency observed by labeled siRNA uptake.

***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

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

***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.

## 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 unlabeled and labeled single-stranded RNA oligonucleotides is used to confirm ≥95% purity and, where applicable, coupling of dye to nucleic acid.

**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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