

Silencer[®] GFP (eGFP) siRNA

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

Catalog #:	AM4626	
Component:	<u>GFP (eGFP) siRNA</u>	<u>Negative Control #1 siRNA</u>
Amount:	5 nmol	2 nmol
Volume:	100 μL	40 μL
Concentration:	50 μM	50 μM

Target Information:	<u>Gene Symbol:</u>	GFP (eGFP)
	<u>Full Gene Name:</u>	Green fluorescent protein
	<u>Organism:</u>	<i>Aequorea victoria</i>
	<u>Acc. No.:</u>	U55761

GFP (eGFP) siRNA targets BD Clontech vectors (all eGFP vectors and core vector pEGFP PT3078-5). This siRNA does not target cycle 3 GFP (found in Invitrogen's pTracer[™] product line).

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:	<p>Ambion <i>Silencer</i>[®] eGFP siRNA can be used to reduce the expression of the eGFP gene (Clontech) in mammalian cells when co-transfected with an eGFP-expressing plasmid or when transfected into cells stably expressing the fluorescent protein. The sense and antisense siRNA strands of the eGFP and Negative Control siRNAs are chemically synthesized, HPLC purified, and then annealed. The siRNAs are provided ready to transfect.</p> <p>The eGFP protein typically reaches its maximum level 24–72h after cells are transiently transfected with a vector expressing this protein, however this time may vary with cell type. <i>Silencer</i>[®] GFP (eGFP) siRNA typically reduces eGFP protein level by 70–95% within 24–48 hours of being transfected.</p> <p>The Negative Control siRNA has no significant homology to any known gene sequences from mouse, rat, or human.</p>
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:	<p>Transfecting siRNAs Into Mammalian Cells</p> <p>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.</p>

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[®] 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.

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 $\geq 95\%$ 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.)
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