

Silencer® siRNA Screening Control Panel

Store at or below -20°C .

Catalog #: AM4640

Product Description: A set of 7 negative control siRNAs and 1 positive control siRNA, designed to enable RNAi researchers to identify the best controls for their cell system.

Content:	Component	Cat #	Amount
	Negative Control #1 siRNA	AM4611	1 nmol
	Negative Control #2 siRNA	AM4613	1 nmol
	Negative Control #3 siRNA	AM4615	1 nmol
	Negative Control #4 siRNA	AM4641	1 nmol
	Negative Control #5 siRNA	AM4642	1 nmol
	Negative Control #6 siRNA	AM4643	1 nmol
	Negative Control #7 siRNA	AM4644	1 nmol
	Silencer® KIF11 (Eg5) siRNA	AM4639	1 nmol

Appearance: Powder

Additional Materials Included: 1.75 mL Nuclease-free Water

Target Information: **KIF11 (Eg5) siRNA**

Gene symbol: KIF11

Full Gene Name: Kinesin family member 11

Organism(s): Human, Mouse and Rat

RefSeq Number(s): NM_004523 (human), NM_010615 (mouse), XM_215287 (rat)

Entrez Gene ID: 3832 (human), 16551 (mouse), 171304 (rat)

Format: Annealed

Purity: HPLC purified

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

General Information:

Silencer® siRNA Screening Control Panel contains seven Negative Control siRNAs that can be used to identify the best control for your cell system. Each Negative Control siRNA was designed to have no significant homology to any known gene sequences from human, mouse, or rat species. The siRNA Screening Control Panel enables RNAi researchers to test multiple Negative Control siRNAs to identify ones that do not induce nonspecific effects on gene expression in their chosen cell system.

In addition, an siRNA targeting Kif11 (Eg5) is included to provide an easy test for transfection efficiency. Knockdown of Kif11 leads to a cytotoxic response which can be visually assessed in many cell types. KIF11 encodes a motor protein that belongs to the kinesin-like protein family involved in chromosome positioning and bipolar spindle formation during cell mitosis. A reduction in KIF11 levels causes mitotic arrest. Cells treated with KIF11 siRNA appear rounded rather than flat, giving a visual indicator of successful siRNA delivery. Examples are available at www.ambion.com/info/kif11

The Negative Control siRNAs have been tested in human and rat cells and shown to have no significant impact on cell proliferation, apoptosis, or cell morphology. The KIF11 mRNA level in transfected and nontransfected cells has been measured by real-time RT-PCR using total RNA isolated 48 hr after transfection. KIF11 siRNA reduced the levels of KIF11 mRNA by 70–95% in every cell line tested.

For each siRNA, the sense and antisense strands are chemically synthesized, HPLC purified, and then annealed. The annealed siRNAs are supplied dried, and Nuclease-free Water is provided for resuspension.

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.

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.

Store the resuspended siRNA at or below –20°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.

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

- Refer to the instructions provided with your transfection agent for the recommended volume.
- 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.
- 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® siRNA Libraries

See www.ambion.com/siRNA

Sets of siRNAs, designed for maximum potency and specificity, to genomes or gene classes.

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.

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).

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 $\geq 95\%$ 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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