

Silencer[®] Select Pre-designed, Validated, and Custom Designed siRNA

Custom Select siRNA

Ambion[®] *In Vivo* Pre-designed, Custom Designed, and Custom siRNA, Standard Purity

Insert PN 4457171 Rev. B

Note: For all reagents, read the Safety Data Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product information

Ambion[®] **Silencer**[®] **Select Pre-designed siRNAs** are designed using a novel algorithm that was developed using the latest advances in machine-learning methods. These next-generation siRNAs exhibit up to 100-fold higher silencing potency than siRNAs from other leading siRNA manufacturers. Off-target activity (assayed by microarray analysis) is reduced by up to 90% because *Silencer* Select siRNAs can be used at 5- to 20-fold lower concentrations, they are bioinformatically screened using the latest knowledge about miRNA seed regions and toxic sequence motifs, and they incorporate strategic chemical modifications. As a result, *Silencer* Select siRNAs provide unrivalled specificity and cleaner, more consistent phenotypic data.

Silencer Select Validated siRNAs have been verified experimentally in cell-based assays to reduce the expression of their individual target genes by at least 80% in at least 3 biological replicates.

Silencer Select Custom Designed siRNAs are designed to your specified target, using the same algorithm and with the same chemical modifications as *Silencer* Select Pre-designed siRNAs.

Custom Select siRNAs are synthesized with your sequence, but they incorporate the same strategic chemical modifications, designed to block off-target activity, that are found in *Silencer* Select siRNAs.

Ambion *In Vivo* Pre-designed siRNAs are designed using the *Silencer* Select algorithm and incorporate additional chemical modifications for superior serum stability (half life >5 hours at 37°C in 90% mouse serum) with *in vivo* applications. Ambion *In Vivo* siRNAs are non-toxic and non-immunogenic *in vitro* (peripheral blood nononuclear cells; PBMC) and *in vivo* (mouse). In cell-based assays, Ambion *In Vivo* siRNAs exhibit potency and specificity equivalent to *Silencer* Select siRNAs.

Ambion *In Vivo* Custom Designed siRNAs are designed to your specified target, using the *Silencer* Select algorithm and Ambion *In Vivo* chemical modifications.

Ambion *In Vivo* Custom siRNAs are synthesized with your sequence, with Ambion *In Vivo* chemical modifications for serum stability.

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.

Storage of dried siRNA: Store at 4 °C, or in a non-frost-free freezer at or below -20 °C (dried oligonucleotides are shipped at ambient temperature). For long-term storage, store at or below -20 °C in a non-frost-free freezer.

Resuspension instructions

For best results, prepare the siRNA stock solution at the highest concentration that is workable for your experiments. As needed, dilute the stock for immediate use.

1. Briefly centrifuge the tube to ensure that the dried siRNA is at the bottom of the tube.
2. Resuspend the 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. A calculator for suspension of dry oligonucleotides is available at:

www4.appliedbiosystems.com/techlib/append/oligo_dilution.html

The resuspended siRNA is ready to transfect at your choice of final concentration.

Storage of resuspended siRNA: Store at or below -20 °C.

siRNA stock solutions at concentrations ≥ 2 µM can undergo up to 50 freeze-thaw cycles without significant degradation. Storage in a frost-free freezer is not recommended, however.

Long-term storage at -70 °C has traditionally been recommended, but siRNA stock solutions at concentrations ≥ 2 µM can be stored at -20 °C for extended periods (up to 1 year).

Guidelines for transfection of mammalian cultured cells

The efficiency with which mammalian cells are transfected with siRNA varies according to the cell type and transfection agent used. To optimize transfection conditions, determine the conditions that result in maximum gene silencing with minimal cytotoxicity.

Once optimal transfection conditions are determined:

- Maintain optimal transfection conditions from experiment to experiment for a given cell type.
- Include controls in all plates for each experiment to ensure consistency.

Determine optimal transfection conditions

Use your experimental cell line, and appropriate positive and negative control siRNAs.

1. Identify an effective transfection agent.
2. Adjust, in order of importance:
 - a. Amount of transfection agent.
 - b. Amount of siRNA.
 - c. Cell density at the time of transfection. In general, 30 to 70% confluency is recommended.

- d. Transfection method (traditional or reverse).
- e. Length of exposure of cells to transfection agent/siRNA complexes.

Many protocols recommend maintaining mammalian cells in the medium used for transfection for 48 hours. We have found that for many *Silencer Select* siRNAs, maximal activity is achieved after 24 hours, and the existing medium can be replaced with fresh medium 24 hours after transfection, resulting in greater viability of the cells.

General transfection starting points

When using *Silencer Select* siRNAs, we suggest starting concentrations of 5- to 20-fold less than typically used for transfection of your experimental cell lines with other siRNAs. We have found that *Silencer Select* siRNAs at final concentrations of 2 to 10 nM reduced mRNA levels >80%, using lipid-mediated transfection in HeLa and U-2 OS human osteosarcoma cells. To increase accuracy and reproducibility when preparing transfection complexes, prepare a dilution of your stock siRNA, and pipet a higher volume of diluted stock.

General transfection starting points for mammalian cells[†]

Plate format	Transfection agent (μL) [‡]	siRNA (pmol) [§]	Cells/well	Total volume/well
96 wells	0.2–1	0.5	6,000	100 μL
24 wells	1–3	2.5	40,000	500 μL
12 wells	2–4	5.0	80,000	1.0 mL
6 wells	3–6	12.5	200,000	2.5 mL

- [†] Appropriate for lipid-mediated reverse transfection and easily transfected cells lines such as HeLa.
- [‡] Lipofectamine[®] RNAiMAX Transfection Reagent recommended. Refer to the instructions provided with your transfection agent for the recommended volume.
- [§] The siRNA amounts indicated result in a final siRNA concentration of 5 nM.

For additional information about siRNA transfection, including transfection conditions for many cell types and optimization protocols, see the siRNA Delivery Resource:

www4.appliedbiosystems.com/techlib/resources/delivery

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