

Silencer® Select Pre-designed and Validated siRNA, In Vivo Ready
Ambion® In Vivo Pre-designed and Custom Designed siRNA, In Vivo Ready or HPLC-purified
Custom Select siRNA and Ambion® In Vivo Custom siRNA, In Vivo Ready

Insert PN 4457172 Rev. C

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. In cell-based assays, 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 bio-informatically 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 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 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.

In Vivo Ready siRNAs are high-quality siRNAs that are purified especially for introduction into animals. Each siRNA strand is individually purified by HPLC, desalted, and annealed with its complementary strand. In Vivo Ready siRNAs are then further purified using a process that removes excess salt via a semi-permeable membrane. The result is a highly pure siRNA with minimal salt content, suitable for in vivo applications. In Vivo Ready siRNAs are then filtered through a 0.2-µm pre-sterilized filter and tested for the presence of endotoxin.

At a concentration of 50 µM in de-ionized water, In Vivo Ready siRNA contains <5.0 mM Na⁺, <0.06 mM K⁺, and <0.02 mM Mg²⁺.

Handling instructions

RNA oligonucleotides such as siRNA 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.
- Use standard biological sterile techniques when handling In Vivo Ready siRNA that will be administered to animals.

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.

Using In Vivo Ready siRNA with InvivoFectamine® 2.0 Reagent

InvivoFectamine® 2.0 Reagent (PN 1388501; www.invitrogen.com) is a proprietary, animal-origin-free, lipid-based transfection reagent that is designed for systemic, in vivo siRNA delivery to mouse liver tissue. InvivoFectamine 2.0 Reagent is ideally suited for use with Ambion *In Vivo* siRNA, with high in vivo transfection efficiency in liver following tail-vein injection. Low volume delivery of siRNA using low pressure, combined with the minimal toxicity of InvivoFectamine 2.0 Reagent, avoids a stress response in the animal.

Resuspension of siRNA for use with InvivoFectamine® 2.0 Reagent

1. Briefly centrifuge the tube to ensure that the dried siRNA is at the bottom of the tube.
2. Resuspend the siRNA in nuclease-free sterile water and vortex to thoroughly resuspend.

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

The working siRNA concentration for use with InvivoFectamine 2.0 Reagent is 200 µM (~3 mg/mL).

A calculator for suspension of dried oligonucleotides is available at: www4.appliedbiosystems.com/techlib/append/oligo_dilution.html

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).

Preparation of Invivofectamine® 2.0 Reagent-siRNA complexes and in vivo delivery

Follow the instructions provided with Invivofectamine 2.0 Reagent, available at the web catalog page at www.invitrogen.com (search for Invivofectamine 2.0 Reagent). An siRNA dose of 7 mg/kg is recommended as a starting point for experiments. This dose corresponds to 200 µL of a 0.7 mg/mL solution injected into a 20-g mouse.

Using In Vivo Ready siRNA with other in vivo delivery strategies

Resuspension of In Vivo Ready siRNA

Follow the instructions for resuspension on [page 1](#). At step 2, resuspend the siRNA in sterile water or a sterile buffer appropriate for your application. Common examples are provided below.

Commonly used sterile buffers for in vivo delivery

Systemic delivery	- Phosphate buffered saline (PBS) - Saline (0.9% NaCl), or variants containing sugars such as mannitol or glucose (5–15%) - Ringer's solution: 147 mM NaCl, 4 mM KCl, 1.13 mM CaCl₂
Central nervous system delivery	- Saline (0.9% NaCl) - Isotonic buffer (100 mM potassium acetate, 30 mM HEPES-KOH, 2 mM magnesium acetate, 26 mM NaCl, pH 7.4)

Store resuspended siRNA as described on [page 1](#).

Suggested dosing for a 20-g mouse

A typical volume for systemic delivery is ~200 µL (for low pressure injection) at concentrations of 50 to 500 µM. This combination corresponds to an siRNA dosing range of 5 to 50 mg/kg for a 20-g mouse.

Dose (mg/kg)	nmol siRNA/Dose	Concentration for 200-µL Dose (µM) [†]
1.0	1.5	7.4
5.0	7.4	37.0
10.0	14.8	74.0
20.0	29.6	148.0
50.0	74.0	370.0

[†] In Vivo Ready siRNAs are soluble in aqueous solution at concentrations ≥ 1 mM.

For more information

Visit these web resources for more information on buffers, injection routes, and dosing strategies for in vivo siRNA delivery:

- www4.appliedbiosystems.com/RNAi/invivo
- www.invitrogen.com ▶ Products & Services ▶ Applications ▶ RNAi, Epigenetics & Gene Regulation ▶ RNAi ▶ in vivo RNAi ▶ in vivo RNAi Protocols

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Safety Data Sheets: Safety Data Sheets (SDSs) are available from www.appliedbiosystems.com/sds.

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