# *Silencer*® GAPDH siRNA, In Vivo Ready

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

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| **Catalog # (P/N):** | 4404025 | |
| **Amount:** | 250 nmol | |
| **Appearance:** | Powder | |
| **Target Information:** | Gene Symbol: | GAPD |
| | Full Gene Name: | Glyceraldehyde-3-phosphate dehydrogenase |
| | Organism(s): | Human, Mouse, and Rat |
| | RefSeq Number(s): | NM_002046 (human), NM_008084.2 (mouse), and NM_017008 (rat) |
| | Entrez Gene ID(s): | 2597 (human), 14433 (mouse), and 24383 (rat) |
| **Format:** | Annealed | |
| **Purity:** | In Vivo Ready (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.) | |
| **Safety Information:** | Read the Safety Data Sheet, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. | |

## USER INFORMATION

**Product Description:**

*Silencer*® GAPDH siRNA, In Vivo Ready, provides a positive control for experiments involving *Silencer* siRNA delivery to animals.

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 utilizing 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 $Mg^{2+}$.

*Silencer* GAPDH siRNA, In Vivo Ready, has the same sequence that has been shown to reduce the expression of GAPDH by 70-95% in several common human, mouse and rat cell lines, and is purified to rigorous In Vivo Ready specifications.

**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, store dried oligonucleotides 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.

Use standard biological sterile techniques when handling In Vivo Ready siRNA that will be administered to animals.

**Using In Vivo Ready siRNA with Invivofectamine® 2.0 Reagent:**

Invivofectamine® 2.0 Reagent (P/N 1388501) is a proprietary, animal-origin-free, lipid-based transfection reagent 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 low 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 is 200 µM (~3 mg/mL).

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

**Storage of resuspended siRNA:** Store at or below –20°C. Stock solutions of siRNA 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-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). 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 with use with Invivofectamine 2.0 Reagent. At step 2, resuspend the siRNA in sterile water or a sterile buffer appropriate for your application. Common examples are provided below.

***Suggested Buffers for Systemic Delivery***

- Sterile, phosphate buffered saline (PBS)
- Sterile 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 $CaCl_2$

***Suggested Non-irritating Buffers for Central Nervous System Delivery***

- Sterile 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 under "Resuspension of siRNA for use with Invivofectamine 2.0 Reagent."

**Suggested dosing for a 20-g mouse**
A typical volume for systemic delivery is ~200 µL (for low pressure injection) at concentrations of 50–500 µM. This combination corresponds to an siRNA dosing range of 5–50 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 up to 1.5 mM.

**For more information**
Visit these web resources for more information on buffers and injection routes:
www4.appliedbiosystems.com/RNAi/invivo

www.invitrogen.com > **Products & Services** > **Applications** > **RNAi, Epigenetics & Gene Regulation** > **RNAi** > **in vivo RNAi** > **in vivo RNAi Protocols**

## RELATED PRODUCTS

**Anti-GAPDH, Mouse Monoclonal 6C5**
P/N AM4300
Ideal for detecting knockdown of GAPDH at the protein level by Western blot or immunofluorescence.

**Ambion® *In Vivo* Pre-designed and Validated siRNAs**
P/N Various (see www4.appliedbiosystems.com/geneassist)
An all-new class of modified siRNAs with superior serum stability for in vivo applications, designed with the *Silencer*® Select algorithm. Ambion® *In Vivo* siRNAs are non-toxic in vivo (mouse) and non-immunogenic (cell-based assays), while exhibiting potency and specificity equivalent to or better than *Silencer* Select siRNAs.

**Invivofectamine® 2.0 Reagent**
P/N 1377-505, 1377-501
A proprietary lipid-based reagent designed to efficiently deliver siRNA to mouse liver tissue in an easy-to-complex preparation. See www.invitrogen.com.

**TaqMan® Gene Expression Assays**
www.allgenes.com or www4.appliedbiosystems.com/geneassist
A comprehensive collection of over 700,000 probe and primer sets for quantitative gene expression analysis using real-time PCR. Search the GeneAssist™ Atlas at www4.appliedbiosystems.com/geneassist to find suggested TaqMan Gene Expression Assays corresponding to your siRNA targets of interest.

## QUALITY CONTROL

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| **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 ≥95% purity. |
| **Annealing:** | A sample of the annealed siRNA is analyzed by nondenaturing gel electrophoresis. |
| **Endotoxin:** | A *Limulus* Amebocyte Lysate (LAL) assay of a sample of the siRNA is used to confirm <10 Endotoxin Units (EU) per nmol. |

## OTHER INFORMATION

**Safety Data Sheets:**

Safety Data Sheets (SDSs; previously known as MSDSs) for any chemical product supplied by Applied Biosystems or Ambion are available 24 hours a day. At www.appliedbiosystems.com, select Support, then SDS/MSDS. Search by chemical name, product name, product part number, or SDS/MSDS part number. Right-click to print or download the SDS of interest. At www.ambion.com, go to the web catalog page for the product of interest. Select SDS/MSDS, then right-click to print or download. Or, e-mail (MSDS_Inquiry_CCRM@appliedbiosystems.com), telephone (650-554-2756; USA), or fax (650-554-2252; USA) your request, specifying the catalog or part number(s) and the name of the product(s). We will e-mail the associated SDSs unless you request fax or postal delivery. Requests for postal delivery require 1-2 weeks for processing.

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***For research use only. Not intended for any animal or human therapeutic or diagnostic use.***

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This product is covered by U.S. Patent Nos. 6,506,559; 7,056,704 and 7,078,196.

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