# Silencer® Select Negative Control siRNA #1 (5 nmol)

**Package Contents**

**Catalog Number** 4390843

**Size** 5 nmol lypohilized pellet

- 1.75 mL Nuclease-free Water

**Storage Conditions**

- Store at or below –20°C. Do not store in a frost-free freezer. (Dried oligonucleotides are shipped at room temperature.)

**Required Materials**

- RNase-free reagents
- Transfection reagent e.g. Lipofectamine® RNAiMAX

**Timing**

Transfection preparation: 15 minutes
Final incubation: 1–3 days

**Selection Guide**

siRNAs
Go online to view related products.

**Product Description**

- Silencer® Select siRNAs are chemically modified, 21-mer, double-stranded RNAs (dsRNAs) with third-generation locked nucleic acid (LNA) chemistry for increased potency and specificity as compared to unmodified 21-mer dsRNAs (Silencer® siRNA).
- Silencer® Select Negative Controls were bioinformatically designed with the latest information about miRNA seed regions and sequence alignment algorithms to minimize interactions with mRNA transcripts in the transcriptomes of human, mouse, and rat. They incorporate LNA chemical modifications for reduced off-target activity and for experimental compatibility with Silencer® Select siRNAs.

**Important Guidelines**

- 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.
- Transfect Silencer® Select Negative Control using the same methodology as for your positive control or experimental siRNAs.
- Transfection efficiency varies according to the cell type and transfection agent used. To optimize, determine the conditions that result in maximum gene silencing with minimal cytotoxicity. Maintain conditions and use positive and negative controls in all plates.

**Online Resources**

Visit our product page for additional information and protocols. For support, visit www.lifetechnologies.com/support.

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Print Options

## siRNA Resuspension Protocol

We recommend preparing 100 µM siRNA stock solution. Dilute the stock solution to 10 µM for immediate use.

1. Briefly centrifuge the tube or plate to ensure that the dried siRNA is at the bottom of the tube.
2. Resuspend the 5 nmol siRNA using 50 µL of the nuclease-free water provided for a final concentration of 100 µM.
3. Make 10 µM working stock using nuclease-free water for immediate use. A 10-µM stock of siRNA duplex is equivalent to 10 pmol/µL.
4. (Optional) Aliquot siRNAs into one or more daughter tubes or plates to limit the number of freeze-thaw cycles to which the siRNAs are subjected. Solutions at concentrations >2 µM can undergo up to 50 freeze-thaw cycles without significant degradation.
5. Store at or below –20°C in a non-frost-free freezer until use.

Once reconstituted in nuclease-free water, the siRNA is ready to transfect and can be used at your choice of final concentration.

## RNAi Transfection Protocol

See page 2 to view guidelines for transfecting siRNAs using Lipofectamine® RNAiMAX Reagent.

## Transfection Amounts per Well

Use 10 nM siRNA duplex as a starting point.

| | 96-well | 24-well | 6-well |
|---|---|---|---|
| Final siRNA | 1 pmol | 5 pmol | 25 pmol |
| Final Lipofectamine® RNAiMAX | 0.3 µL | 1.5 µL | 7.5 µL |

## Reverse Transfection of RNAi

Reverse transfection is faster to perform than forward transfection and is the method of choice for high-throughput transfection. Perform reverse transfection by preparing the siRNA transfection complexes inside the wells, and then adding cells and medium. Because the cells and siRNA-reagent complexes are prepared on the same day, we recommended using 2.5× more cells than for a regular transfection.

## Limited Product Warranty and Disclaimer Details

## Limited Use Label Licenses

## Guarantee

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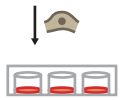
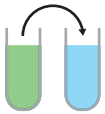
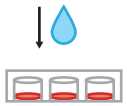
# RNAi Transfection Protocol

This procedure is designed for one RNA amount combined with one amount of Lipofectamine® RNAiMAX.
**The prepared mix is enough to have triplicates (96-well), duplicates (24-well), and single well (6-well) transfections, and account for pipetting variations.**

| Timeline | | Steps |
|---|---|---|
| Day 0 | 1  | **Seed cells to be 60-80% confluent at transfection** |
| | 2  | **Dilute Lipofectamine® RNAiMAX Reagent in Opti-MEM® Medium** |
| | 3  | **Dilute siRNA in Opti-MEM® Medium** |
| Day 1 | 4  | **Add diluted siRNA to diluted Lipofectamine® RNAiMAX Reagent (1:1 ratio)** |
| | 5  | **Incubate** |
| | 6  | **Add siRNA-lipid complex to cells** |
| Day 2–4 | 7  | **Visualize/analyze transfected cells** |

**Procedure Details**

| Component | 96-well | 24-well | 6-well |
|---|---|---|---|
| Adherent cells | 1–4 × $10^4$ | 0.5–2 × $10^5$ | 0.25–1 × $10^6$ |
| Opti-MEM® Medium | 25 µL | 50 µL | 150 µL |
| Lipofectamine® RNAiMAX Reagent | 1.5 µL | 3 µL | 9 µL |
| Opti-MEM® Medium | 25 µL | 50 µL | 150 µL |
| siRNA (10 µM) | 0.5 µL (5 pmol) | 1 µL (10 pmol) | 3 µL (30 pmol) |
| Diluted siRNA | 25 µL | 50 µL | 150 µL |
| Diluted Lipofectamine® RNAiMAX Reagent | 25 µL | 50 µL | 150 µL |
| Incubate for 5 minutes at room temperature. | | | |
| **Component** | **96-well** | **24-well** | **6-well** |
| siRNA-lipid complex per well | 10 µL | 50 µL | 250 µL |
| Final siRNA used per well | 1 pmol | 5 pmol | 25 pmol |
| Final Lipofectamine® RNAiMAX used per well | 0.3 µL | 1.5 µL | 7.5 µL |
| Incubate cells for 1–3 days at 37°C. Then, analyze transfected cells. | | | |