Silencer® Select Negative Control siRNA #1 (40 nmol)

Package Contents

Catalog Number	Size
4390844	40 nmol lyophilized 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 40 nmol siRNA using 400 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 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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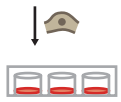
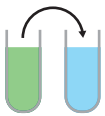
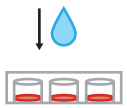
RNAi Transfection Protocol

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.			