

siLentMer™ Delivery Optimization Kit

174-9950

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Storage and Stability

siRNA: lyophilized duplexes are guaranteed stable for 1 year from date of purchase when stored at 4°C or –20°C. Resuspended siLentMer siRNA should be stored at –20°C. Fluorescently labeled siRNA should be protected from prolonged exposure to light.

Lipid: siLentFect lipid reagent should be stored at 4°C. **Do not store below 0°C.**

Buffer: siLentMer siRNA resuspension buffer can be stored at room temperature to –20°C.

Handling Precautions

RNA oligonucleotides are susceptible to degradation by RNases. Use gloves and RNase-free reagents, pipets, and tubes.

Overview

siLentMer delivery optimization kit provides a set of reagents to facilitate transfection optimization for cell lines to be used in RNAi experiments. This kit contains a nonsilencing siRNA labeled with CAL Fluor Red 610, siLentFect lipid reagent, and siRNA resuspension buffer. The fluorescently labeled nonsilencing siRNA enables visual monitoring of transfection efficiency during RNAi experiments.

Once optimal transfection conditions are determined, positive and negative siRNA controls should be established before moving on to silencing your genes of interest. For a list of kits with controls and a current list of validated gene targets, go to www.bio-rad.com/RNA.

Kit Contents*

Reagent	Amount/ Volume	Description
siLentMer fluorescently labeled nonsilencing siRNA (control for delivery) – <i>amber vial/ cap</i>	1.0 nmol (lyophilized)	nonsilencing siRNA, fluorescently labeled (CAL Fluor Red 610, absorbance max 590 nm, emission max 610 nm)
siLentFect lipid reagent – <i>white label</i>	0.2 ml	cationic lipid transfection reagent
siLentMer siRNA resuspension buffer – <i>purple label</i>	1.0 ml	100 mM potassium acetate, 30 mM HEPES, pH 7.5

*Using a 24-well format, this kit is sufficient for approximately 300 reactions using 5 nM siRNA per transfection.

siLentMer Resuspension Protocol for Vials Containing 1.0 nmol Lyophilized siRNA

1. Centrifuge the tube prior to opening. Some of the product may have been dislodged during shipping. To create a 10 µM stock, add 100 µl of siRNA resuspension buffer to the vial. Mix contents by vortexing.
2. Incubate tube at 94°C for 2 minutes. Remove from heat and allow tube to cool to room temperature (15–30 minutes).
3. Once resuspended, the siRNA is ready to use. Store at –20°C.
4. To avoid multiple freeze/thaw cycles, divide the content into smaller aliquots and store at –20°C.
5. Protect fluorescently labeled siRNA from excessive exposure to light.

Recommended for Optimal Transfection Conditions

For every cell type used, it is important to experimentally determine optimal transfection conditions to maximize gene silencing. To achieve maximum transfection efficiency with minimum toxicity the following parameters need to be optimized: amount of transfection reagent, concentration of siRNA, cell density, transfection method, and cell incubation time with complexes. For additional details see the siLentFect transfection protocol.

Sample Protocol

Transfection

1. For a 24-well transfection, dilute siRNA to a final volume of 25 μ l in serum-free medium. As a starting point use a final siRNA concentration of 5 nM.
2. Dilute siLentFect lipid reagent to a final volume of 25 μ l in serum-free medium. When optimizing transfection efficiency, use a constant siRNA concentration and vary the amount of siLentFect from 0.25 to 1.0 μ l. See Table 1 below for some suggested conditions.
3. Combine and mix diluted siRNA and diluted lipid. Incubate for 20 min at room temperature.
4. Add siRNA-lipid complexes to cells, and disperse by rocking the plate.

Detection

5. Transfection efficiency can be monitored from 4 to 72 hr posttransfection using the fluorescently labeled siRNA and a fluorescence microscope.

Table 1. Suggested Reagent Quantities for Different Sizes of Plates/Wells

Culture Vessel Size	Volume of Plating Medium (ml)	siRNA conc.* (nM)	Volume of Serum-Free Medium (μ l)	siLentFect Reagent* (μ l)
96 well	0.5	5–20	20	0.05–0.4
24 well	0.5	5–20	50	0.25–2.0
6 well	2.5	5–20	250	1.0–5.0
100 mm	10	5–20	1,000	5.0–20.0

*As a starting point, use 5 nM siRNA and a range of siLentFect volumes.

Quality Control

The siLentMer Dicer-substrate siRNA duplexes are quality-controlled by HPLC. The identity of the duplex is confirmed by electrospray ionization mass spectrometry (ESI-MS)

Ordering Information

Catalog # Description

siLentMer Total Control Kits

174-9970	siLentMer Total Control Kit for Human GAPDH
174-9971	siLentMer Total Control Kit for Human HPRT
174-9972	siLentMer Total Control Kit for Human Lamin A/C
174-9973	siLentMer Total Control Kit for Human Cyclophilin
174-9974	siLentMer Total Control Kit for Human β-Actin
174-9975	siLentMer Total Control Kit for Human β-Tubulin
174-9976	siLentMer Total Control Kit for GFP
174-9977	siLentMer Total Control Kit for Luciferase

Visit www.bio-rad.com/RNAi/ for a complete list of validated and predesigned siRNA duplexes and kits



For custom 27-mer siRNA synthesis, please see our siRNA content partner, Integrated DNA Technologies, Inc. at www.idtdna.com. The Dicer-substrate siRNA duplexes are manufactured for Bio-Rad by Integrated DNA Technologies, Inc.

CAL Fluor Red is a trademark of Biosearch Technologies, Inc.