siLentFect™ Lipid Reagent

0.2 ml	170-3360S
0.5 ml	170-3360
1.0 ml	170-3361
5 x 1.0 ml	170-3362

For Research Use Only
Store at 4°C

Storage and Stability

siLentFect Lipid Reagent is shipped on ice. Store at 4ºC upon receipt. Do not store below 0ºC. siLentFect is stable for 6 months from date of purchase when stored at 4ºC.

Contents

siLentFect contains 0.2 ml (Catalog #170-3360S), 0.5 ml (Catalog #170-3360), 1.0 ml (Catalog #170-3361), or 5 x 1 ml (Catalog #170-3362) of a proprietary lipid formulation. One milliliter is generally sufficient for 200 to 500 cell transfections in 35 mm plates. siLentFect comes as a 1mg/ml solution.

Typically, siLentFect requires less reagent than other lipids for effective siRNA delivery. When working with different lipids or new cell lines, it is important to perform a dilution series of both siRNA and lipids to ensure optimal results. Please see Table 1 for suggested reagent and siRNA concentrations.

Overview

RNA interference (RNAi) is a powerful tool used to manipulate gene expression and allow researchers to determine gene function. RNAi is the sequence-specific gene silencing induced by double-stranded RNAs. Small interfering RNAs (siRNAs) are double-stranded RNA molecules, 21 nucleotides in length, that greatly stimulate gene-specific silencing. siLentFect has been developed to produce exceptional delivery of siRNA to mammalian cells in culture.

siLentFect Lipid Reagent is a mixture of a proprietary cationic compound and a co-lipid. These compounds have been optimized for the intracellular delivery of siRNAs into cultured mammalian cells in the presence of serum at cell densities from 50% to 70%. For most cell lines, high levels of gene silencing can be obtained using amounts of siLentFect and siRNA suggested in this manual for the stated culture sizes. **For best results it is important to determine the optimal amount of siRNA and lipid for any given cell line**.

Recommendations for Optimal Results

siLentFect has been developed to achieve consistent transfection efficiencies using a broad range of cell types with an easy-to-use protocol. Optimum transfection efficiencies are achieved by adjusting:

- Quantities of siLentFect reagent
- siRNA concentration
- Cell density at the time of transfection
- Length of exposure of cells to siLentFect-siRNA complexes

Once maximum transfection efficiency has been established, the conditions should be kept constant between experiments for any particular cell line.

- Invert the tube of siLentFect to mix contents before using.
- Use sterile polystyrene plasticware (e.g. 12 x 75 mm tubes or multi-well trays) to prepare the siRNA solutions and lipid solutions. Polystyrene is recommended because cationic lipid-siRNA complexes may bind to polypropylene.

Optimization

Determining the optimum conditions for transfection efficiency is essential to maximize gene silencing and to minimize cellular toxicity. The two most important parameters to optimize for any given culture vessel and cell density are the amount of siLentFect and the concentration of siRNA. **See Table 1 for suggested reagent and siRNA concentrations for different culture vessels**. If toxicity is encountered try reducing lipid amounts used in transfection. The amount of siLentFect and the concentration of siRNA required for maximal gene silencing can vary among different cell types.

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

Culture Vessel Size	Volume of Plating Media	siRNA conc.	Volume of Serum Free Medium	siLentFect Reagent
96 well	0.1 ml	5–20 nM	20 µl	0.05–0.4 µl
24 well	0.5 ml*	5–20 nM	50 µl	0.25–2.0 µl
12 well	1.0 ml*	5–20 nM	100 µl	0.5–4.0 µl
6 well/35mm	2.5 ml*	5–20 nM	250 µl	1.0–5.0 µl
60 mm	5.0 ml*	5–20 nM	500 µl	2.5–10 µl
100 mm	10.0 ml*	5–20 nM	1.0 ml	5.0–20 µl

*Carefully aspirate medium 15-60 minutes prior to transfection and add one-half volume of medium.

Typically, siLentFect requires less reagent than other lipids for effective siRNA delivery. When working with different lipids or new cell lines, it is important to perform a dilution series of both siRNA and lipids to ensure optimal results. Please see Table 1 for suggested reagent and siRNA concentrations.

Protocol for Transfection of Adherent Cells
(24 Well Plates)

1. The day before transfection, inoculate 24-well plates with an appropriate number of cells in serum-containing medium such that they will be 50 to 70% confluent the following day. For most cell lines, we recommend plating between 0.1 and 4.0 x 10^5 cells in 0.5 ml of medium. Incubate the cells at 37° C in a 5% CO_2 incubator overnight.
2. Fifteen to sixty minutes prior to transfection, carefully aspirate the medium from the wells and add 250 µl of fresh growth medium to each well.
3. For each well to be transfected, prepare 25 µl of serum-free medium containing 0.75 µl of siLentFect as a starting point. The optimal amount of siLentFect can be determined by using the range recommended in Table 1 (0.25 µl to 2 µl).
4. For each well to be transfected, prepare 25 µl of serum-free medium containing siRNA. Use a final concentration of 10 nM as a starting point. For example, for a 24-well plate with 250 µl of growth medium per well, prepare 25 µl of serum-free medium containing 120 nM of siRNA. After mixing with the diluted siLentFect from step 3 and addition to cells, the final concentration will be 10 nM. The optimal concentration of siRNA may vary from 5 to 20 nM depending on the cell line used and the gene to be targeted.
5. Add the diluted siRNA to the diluted siLentFect. Mix by tapping or pipetting. Incubate 20 minutes at room temperature.
6. Add 50 µl of complexes directly to cells in serum-containing medium. Rock the plate back and forth to mix. Incubate the cells at 37°C in a CO_2 incubator.

Alternative method

The siLentFect /siRNA complexes can be added directly to the cells without changing the growth medium. However, to achieve a final concentration of 10 nM siRNA in a well with 500 µl of growth medium, prepare 25 µl of serum-free medium containing 240 nM of siRNA. After mixing the diluted siRNA with siLentFect and addition to cells the final concentration will be 10 nM.

7. Gene silencing can be monitored at the mRNA or protein levels from 4 to 72 hours after the transfection. If toxicity is a problem, change the medium 4–24 hours post transfection.

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