siRNA transfection in HUVEC cells.

1. Make the stock solution of siRNA (10um). Dilute 2nm siRNA into 200ul RNAase free water to make 10um of stock solution.

2. To test the knockdown efficiency, Transfer 2ul (10nm final concentration) or 4ul (20nm final concentration) siRNA stock solution to 100ul of Opti-MEM and mix it, which is for one well of 6-well plate.

3. Dilute 4ul siLentFect Reagent/well into 100ul of opti-MEM and immediately mix with 100ul siRNA-optiMEM medium prepared above. Let sit at RT for 20min.

4. At the same time, change the HUVEC EGM2 medium to fresh one.

5. Pour the ready to go silentFect-siRNA to HUVEC cells and incubate for 2days. The medium can be changed one days after the transfection, but it is not necessary since the toxicity of siLentFect is pretty low.

6. Two days afterwards transfection, collect the cell by using RLT buffer on plate for RNA extraction.

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.