QIAGEN Supplementary Protocol

Transfection of adherent cells with low concentrations of siRNA using RNAiFect® Transfection Reagent

Some RNAi applications require the use of low amounts of siRNA. This supplementary protocol is a modified version of the RNAiFect Transfection Reagent protocol (see *RNAiFect Transfection Handbook*), that is optimized for transfection of adherent cells with low siRNA concentrations. This protocol has been tested for many commonly used cell lines including HeLa, HeLa S3, HEK 293, MCF-7, and HepG2. We recommend that initial experiments should be carried out with an siRNA concentration of 30 nM. The ratio of RNAiFect Reagent to siRNA and the final siRNA concentration should then be optimized from this starting point.

This supplementary protocol is for transfection of adherent cells in a single well of a 24-well plate. It is provided as a starting point for optimization of siRNA transfection in mammalian cells. A pipetting scheme for optimizing siRNA transfection in a 24-well plate is shown in Table 1. Starting points for optimizing the transfection of adherent cells in other formats are shown in Table 2.

IMPORTANT: Please consult the "Safety Information" and "Important Notes" sections in the *RNAiFect Transfection Handbook* before beginning this procedure.

Equipment and reagents

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Culture medium
- siRNA of interest. Visit www.qiagen.com/siRNA for more information about ordering.

Procedure

1. **The day before transfection, seed 4–8 x 10^4 cells (depending on the cell type and time of analysis) per well of a 24-well plate in 0.5 ml of an appropriate culture medium containing serum and antibiotics.**
2. **Incubate cells under normal growth conditions (typically 37°C and 5% CO_2).**
3. **On the day of transfection, dilute 0.3 µg siRNA (corresponding to 30 nM final concentration) in the appropriate volume of Buffer EC to give a final volume of 100 µl, and mix by vortexing.**
4. **For complex formation, add 3.6 µl of RNAiFect Transfection Reagent to the diluted siRNA. Mix by pipetting up and down 5 times, or by vortexing for 10 s.**
5. **Incubate the samples for 10–15 min at room temperature (15–25°C) to allow formation of transfection complexes.**
6. **While complex formation is taking place, gently aspirate the culture medium from the plate. Add 700 µl of fresh culture medium containing serum and antibiotics to the cells.**
7. **Add the complexes drop-wise onto the cells. Gently swirl the plate to ensure uniform distribution of the transfection complexes.**
8. **Incubate cells with the transfection complexes under their normal growth conditions and monitor gene silencing at the mRNA or protein level after an appropriate time.**

Table 1. Pipetting scheme for optimizing siRNA transfection of adherent cells in a 24-well plate*

	Ratio of siRNA to RNAiFect Reagent (µg:µl)		
	1:6	1:12	1:24
Amount of siRNA	0.3 µg (30 nM)	0.3 µg (30 nM)	0.3 µg (30 nM)
Volume of RNAiFect Reagent	1.8 µl	3.6 µl	7.2 µl
Amount of siRNA	0.2 µg (20 nM)	0.2 µg (20 nM)	0.2 µg (20 nM)
Volume of RNAiFect Reagent	1.2 µl	2.4 µl	4.8 µl
Amount of siRNA	0.1 µg (10 nM)	0.1 µg (10 nM)	0.1 µg (10 nM)
Volume of RNAiFect Reagent	0.6 µl	1.2 µl	2.4 µl

* Amounts given are per well of a 24-well plate

Table 2. Starting points for optimizing the transfection of adherent cells in different formats

Culture format	siRNA, µg	Volume of 20 µM siRNA stock, µl	Final volume of diluted siRNA, µl	RNAiFect Reagent, µl	Volume of medium on cells, µl	Final siRNA conc., nM
Protocol step	3	3	3	4	6	
96-well plate	0.075	0.3	25	0.9	175	30
48-well plate	0.15	0.6	50	1.8	350	30
24-well plate	0.3	1.2	100	3.6	700	30
12-well plate	0.6	2.4	100	7.2	1500	30
6-well plate	1.2	4.8	100	14.4	3000	30

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Safety data sheets (SDS) for any QIAGEN product can be downloaded from www.qiagen.com/safety.

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