Experimental Procedures

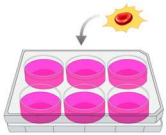
Steps		Procedure Details
Resuspension Protocol		
1	 **Resuspension of siRNA**	1. Briefly centrifuge tubes (or multi-well plates) containing siRNA to ensure that the siRNA pellet is located at the bottom of the tube. 2. Dissolve siRNAs to a convenient stock concentration using the recommended volume of DEPC-D.W. (or RNase-free water) shown in Table 1. 3. Mix the siRNAs by pipetting or vortexing briefly and spin down. 4. Store at -20°C in small aliquots and avoid repeated freeze and thaw cycles.

Amount of siRNA (nmol)	Volume of DEPC-D.W. for desired final concentration	
	100 µM stock	20 µM stock
10	100 µl	500 µl
20	200 µl	1000 µl
50	500 µl	Exceeds tube volume
100	1000 µl	

Table 1. Recommended volumes and concentrations for siRNA resuspension.

Transfection Protocol

- We used Lipofectamine® RNAiMAX (Thermo Fisher) and a HeLa cell for the validation of our siRNA.
- This protocol is optimized for transfection in a 6-well culture plate format.
- To perform transfection in different cell culture formats, refer to Table 2 and the manufacture's Lipofectamine® RNAiMAX protocol.

1	 **Preparation of cells**	1. One day before transfection, plate 0.25-1 X 10^6 cells (adherent cells) in each well with 2 ml of growth medium without antibiotics so that they will be 60-80% confluent at the time of transfection.

Culture Vessel	Relative surface area*	Volume of plating medium
96-well	0.2 cm^2	100 µl
48-well	0.4 cm^2	250 µl
24-well	1 cm^2	500 µl
6-well	5 cm^2	2 ml
60 mm	10 cm^2	5 ml
100 mm	30 cm^2	10 ml

Table 2. Relative surface area of *in vitro* cell culture dish and culture media volume.
* Surface area may vary according to manufacturers.

2. Remove the growth medium from the cells.
3. Add 500 µl of fresh growth medium without serum.

2	 **Preparation of mixture**	4. For each well to be transfected, prepare siRNA-Lipofectamine® RNAiMAX complexes as follows. 4-1. Dilute 3 µl of siRNA (10 µM stock) in 150 µl of growth medium without serum (or Opti-MEM® I Reduced Serum medium) and mix gently. 4-2. Prepare diluted Lipofectamine® RNAiMAX before use. Add 9 µl of Lipofectamine® RNAiMAX in 150 µl of growth medium without serum (or Opti-MEM® I Reduced Serum medium). Incubate for 5 min at room temperature. 4-3. Combine the diluted siRNA duplex with diluted Lipofectamine® RNAiMAX (1:1 ratio). Gently mix and incubate for 20 min at room temperature.
3	 **Add mixture and incubate cells**	5. Add 250 µl of the mixture (siRNA duplex with Lipofectamine® RNAiMAX) to each well of 6-well plate containing cells. The final volume in each well is 750 µl. The amount of siRNA used per well is 25 pmol. Mix gently with hands by rocking the plate back and forth. 6. Incubate the cells for 5-6 hrs at 37°C in CO_2 incubator.
4	 **Analyze transfected cells**	7. Change the medium with a fresh one containing serum and incubate the cells 24-48 hrs until you are ready to analyze for siRNA functional studies.