
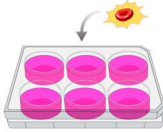





Experimental Procedures

Steps		Procedure Details																					
Resuspension Protocol																							
1	 <p>Resuspension of siRNA</p>	<ol style="list-style-type: none"> Briefly centrifuge tubes (or multi-well plates) containing siRNA to ensure that the siRNA pellet is located at the bottom of the tube. Dissolve siRNAs to a convenient stock concentration using the recommended volume of DEPC-D.W. (or RNase-free water) shown in Table 1. Mix the siRNAs by pipetting or vortexing briefly and spin down. Store at -20°C in small aliquots and avoid repeated freeze and thaw cycles. <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th rowspan="2">Amount of siRNA (nmol)</th> <th colspan="2">Volume of DEPC-D.W. for desired final concentration</th> </tr> <tr> <th>100 µM stock</th> <th>20 µM stock</th> </tr> </thead> <tbody> <tr> <td style="text-align: center;">10</td> <td style="text-align: center;">100 µl</td> <td style="text-align: center;">500 µl</td> </tr> <tr> <td style="text-align: center;">20</td> <td style="text-align: center;">200 µl</td> <td style="text-align: center;">1000 µl</td> </tr> <tr> <td style="text-align: center;">50</td> <td style="text-align: center;">500 µl</td> <td style="text-align: center;">Exceeds tube volume</td> </tr> <tr> <td style="text-align: center;">100</td> <td style="text-align: center;">1000 µl</td> <td style="text-align: center;">Exceeds tube volume</td> </tr> </tbody> </table> <p>Table 1. Recommended volumes and concentrations for siRNA resuspension.</p>	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	Exceeds tube volume				
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Transfection Protocol																							
<ul style="list-style-type: none"> 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	 <p>Preparation of cells</p>	<ol style="list-style-type: none"> One day before transfection, plate 0.25-1 X 10⁶ 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. <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Culture Vessel</th> <th>Relative surface area*</th> <th>Volume of plating medium</th> </tr> </thead> <tbody> <tr> <td style="text-align: center;">96-well</td> <td style="text-align: center;">0.2 cm²</td> <td style="text-align: center;">100 µl</td> </tr> <tr> <td style="text-align: center;">48-well</td> <td style="text-align: center;">0.4 cm²</td> <td style="text-align: center;">250 µl</td> </tr> <tr> <td style="text-align: center;">24-well</td> <td style="text-align: center;">1 cm²</td> <td style="text-align: center;">500 µl</td> </tr> <tr> <td style="text-align: center;">6-well</td> <td style="text-align: center;">5 cm²</td> <td style="text-align: center;">2 ml</td> </tr> <tr> <td style="text-align: center;">60 mm</td> <td style="text-align: center;">10 cm²</td> <td style="text-align: center;">5 ml</td> </tr> <tr> <td style="text-align: center;">100 mm</td> <td style="text-align: center;">30 cm²</td> <td style="text-align: center;">10 ml</td> </tr> </tbody> </table> <p>Table 2. Relative surface area of <i>in vitro</i> cell culture dish and culture media volume.</p> <p>* Surface area may vary according to manufacturers.</p> <ol style="list-style-type: none"> Remove the growth medium from the cells. Add 500 µl of fresh growth medium without serum. 	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
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<p>2</p>	 <p>Preparation of mixture</p>	<p>4. For each well to be transfected, prepare siRNA-Lipofectamine® RNAiMAX complexes as follows.</p> <p>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.</p> <p>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.</p> <p>4-3. Combine the diluted siRNA duplex with diluted Lipofectamine® RNAiMAX (1:1 ratio). Gently mix and incubate for 20 min at room temperature.</p>
<p>3</p>	 <p>Add mixture and incubate cells</p>	<p>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 1 ml. The amount of siRNA used per well is 25 pmol. Mix gently with hands by rocking the plate back and forth.</p> <p>6. Incubate the cells for 5-6 hrs at 37°C in CO₂ incubator.</p>
<p>4</p>	 <p>Analyze transfected cells</p>	<p>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.</p>