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**Bioneer siRNA User MANUAL****1. Dilution Protocol**

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-DW (or RNase-free water) shown in Table 1.
3. Pipette the solution up and down 3-5 times (or vortex briefly).
4. Briefly centrifuge tubes (or multi-well plates) containing siRNA to ensure that the solution is collected at the bottom of the tube.
5. Aliquot the siRNAs into small volumes and store at -20°C. siRNA is stable for 1 year under the specified storage condition. For best results, limit freeze-thaw events for each tube no more than five.

Table 1. Recommended siRNA Resuspension Volumes and concentrations

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

**2. Transfection Protocol**

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\* We use the Lipofectamine™ RNAiMAX (invitrogen; Cat.No. 13778) and HeLa cell for transfection procedure.

\* This protocol is fixed at 6-well plate in vitro culture condition (if you want to change this condition, you have to consider the relative surface area (table 2) and invitrogen protocol, when you are seeding the cells into the culture dish).

1. One day (24 hours) before transfection, plate  $3.0 \times 10^5$  HeLa cells in each well with 2.5ml of growth medium without antibiotics such that they will be 50-60% confluent at the time of transfection.
2. Remove the growth medium from the 6-well plate before transfection. And add the 500µl fresh growth medium without serum in each well.
3. For each well to be transfected, prepare siRNA duplex-Lipofectamine™ RNAiMAX complexes as follows.
  - 3-1. Dilute siRNA duplex (making final concentration as 5nM-100nM) in 250µl growth medium (or Opti-MEM® I Reduced Serum medium) without serum. Mix gently by vortex.
  - 3-2. Mix Lipofectamine™ RNAiMAX gently before use, then dilute 3.5µl in 250µl medium (or Opti-MEM® I Reduced Serum medium) without serum. Incubate this solution 5 minutes at room temperature.
  - 3-3. Combine the diluted siRNA duplex with the diluted Lipofectamine™ RNAiMAX. Mix and incubate for 20 minutes at room temperature.
4. Add the mixture to each well containing HeLa cells, which result 1ml as total volume. Mix gently by hand rocking the plate back and forth.
5. Incubate the cells for 5-6 hours at 37°C in CO2 incubator.
6. Change the medium with fresh one containing serum and incubate the cells 24-48 hours until you are ready to assay for gene knockdown.

Table 2. The relative surface area of in vitro cell culture dish and culture media volume

Culture Vessel	Relative surface area	Volume of plating medium
96-well	0.2	100µl
48-well	0.4	200µl
24-well	1	500µl
6-well	5	2.5ml
60mm	10	5ml
100mm	30	10ml