Transfecting siRNA using Lipofectamine™ RNAiMAX

Pin Liu, 2017

Declaration: The protocal is from Thermo Fisher Scientific

Important Guidelines for Transfection

1. For the cell lines easy to transfection, you can use either of the procedures (**Reverse or Forward Transfection**); For the hard-to-transfect-cell line (such as HepG2), we recommend the **Reverse Transfection.**
2. **Do not** add antibiotics to media during transfection as this causes cell death.
3. Lipofectamine ™ RNAiMAX has a broad peak of activity; for a range of cell densities and volumes of transfection reagent suitable for use, see Acceptable Range for Maximal Activity (page 3)

Reagents:

Opti-MEM I Reduced Serum Medium

Lipofectamine® RNAiMAX Transfection Reagent Thermo Fisher Scientific (13778150)

siRNA (10 µm/L) of interest

serum-free cell culture media

Forward Transfection

Use this procedure to forward transfect siRNA into mammalian cells in a **24-well format** (for other formats, see Scaling Up or Down Transfections, page 3). In forward transfections, cells are plated in the wells, and the transfection mix is generally prepared and added the next day. All amounts and volumes are given on a per well basis.

1. One day before transfection, plate cells in 500 µl of growth medium without antibiotics such that they will be 30-50% confluent at the time of transfection.

2. For each well to be transfected, prepare RNAi duplex-Lipofectamine ™ RNAiMAX complexes as follows:

a. Dilute 6 pmol RNAi duplex in 50 µl Opti-MEM ® I Reduced Serum Medium without serum. Mix gently.

b. Mix Lipofectamine ™ RNAiMAX gently before use, then dilute 1 µl in 50 µl Opti-MEM ® I Reduced Serum Medium. Mix gently.

c. Combine the diluted RNAi duplex with the diluted Lipofectamine ™ RNAiMAX. Mix gently and incubate for 10-20 minutes at room temperature.

3. Add the RNAi duplex-Lipofectamine ™ RNAiMAX complexes to each well containing cells. This gives a final volume of 600 µl and a final RNA concentration of 10 nM. Mix gently by rocking the plate back and forth.

4. Incubate the cells 24-48 hours at 37°C in a CO2 incubator until you are ready to assay for gene knockdown. Medium may be changed after 4-6 hours.

Reverse Transfection

Use this procedure to reverse transfect Stealth ™ RNAi or siRNA into mammalian cells in a 24-well format (for other formats, see Scaling Up or Down Transfections, page 3). In reverse transfections, the complexes are prepared inside the wells, after which cells and medium are added. Reverse transfections are faster to perform than forward transfections, and are the method of choice for high-throughput transfection. All amounts and volumes are given on a per well basis.

1. For each well to be transfected, prepare RNAi duplex-Lipofectamine ™ RNAiMAX complexes as follows.

a. Dilute 6 pmol RNAi duplex in 100 µl Opti-MEM ® I Medium without serum in the well of the tissue culture plate. Mix gently.

b. Mix Lipofectamine ™ RNAiMAX gently before use, then add 1 µl Lipofectamine ™ RNAiMAX to each well containing the diluted RNAi molecules. Mix gently and incubate for 10-20 minutes at room temperature.

2. Dilute cells in complete growth medium without antibiotics so that 500 µl contains the appropriate number of cells to give 30-50% confluence 24 hours after plating. Use 20,000-50,000 cells/well for suspension cells.

3. To each well with RNAi duplex - Lipofectamine ™ RNAiMAX complexes, add 500 µl of the diluted cells. This gives a final volume of 600 µl and a final RNA concentration of 10 nM. Mix gently by rocking the plate back and forth.

4. Incubate the cells 24-72 hours at 37°C in a CO_2 incubator until you are ready to assay for gene knockdown.

Scaling Up or Down Transfections

To transfect cells in different tissue culture formats, vary the amounts of Lipofectamine ™ RNAiMAX, RNAi duplex, cells, and medium used in proportion to the relative surface area, as shown in the table.

Culture vessel	Rel. surf. area	Volume of plating medium	Cells plated per well		Dilution medium		siRNA(pmol) (**Recommed*)**	Lipofect-amine RNAiMAX (**Recommed*)**)
			Start point	Acceptable range	Reverse (ul)	Forward (ul)		
96-well	0.2	100ul	10000	7500-15000	20 µl	2 x 10 µl	0.12-6	0.1-0.3 µl
48-well	0.4	200ul	20000	15000-30000	40 µl	2 x 20 µl	0.24-12	0.2-0.6 µl
24-well	1	500ul	50000	40000-75000	100 µl	2 x 50 µl	0.6-30 (6)	0.5-1.5 µl (1)
6-well	5	2.5ml	250000	200000-375000	500 µl	2 x 250 µl	3-150 (30)	2.5-7.5 µl (5)
60mm	10	5ml			1ml	2 x 500 µl	6-300	5-15 µl
100mm	30	10ml			2ml	2 x 1 ml	12-600	15-35 µl

Recommend* shows the dose we already validated in some HCC and HB cell lines ,such as HLE, SNU449,SNU475,HepG2, Hep293TT