Saatcioglu's Lab Protocol #xxxx

Forward Transfection Using Lipofectamine® RNAiMAX

Use this procedure to forward transfect Stealth™ RNAi or siRNA into mammalian cells in a 6- well format (for other formats, see Scaling Up or Down Transfections Table) . 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.

Reagents:

Lipofectamine® RNAiMAX (Thermo Fisher Scientific # 13778150); RNAi duplex solution; Sterile tubes;

Procedures:

1. One day before transfection, plate cells in 2 ml of growth medium without antibiotics such that they will be 50-60% confluent at the time of transfection.
2. For each well to be transfected, prepare RNAi duplex-Lipofectamine® RNAiMAX complexes as follows:
    - Dilute 20 pmol RNAi duplex in 50 µl Opti-MEM® I Reduced Serum Medium without serum. Mix gently.
    - Mix Lipofectamine™ RNAiMAX gently before use, then dilute 1 µl in 50 µl Opti-MEM® I Reduced Serum Medium. Mix gently.
    - Combine the diluted RNAi duplex with the diluted Lipofectamine™ RNAiMAX. Mix gently and incubate for 10 minutes (no more than) at room temperature.
3. Add the RNAi duplex-Lipofectamine® RNAiMAX complexes (100 µl) to each well containing cells. This gives a final RNA concentration of ~10 nM per well. Mix gently by rocking the plate back and forth.
4. Incubate the cells 48-72 hours at 37°C in a CO_2 incubator until you are ready to assay for gene knockdown. Medium may be changed after 4-6 hours.

Table: Scaling Up or Down Transfections for Various Formates

Components	10-cm	6-cm	6-well	12-well	24-well	96-well	Note
Lipofectamine® RNAiMAX (µl)	5 µl	2 µl	1 µl	0.4 µl	0.2 µl	0.05 µl	
Opti-MEM® I Medium for Lipo dilution	200 µl	100 µl	50 µl	20 µl	10 µl	2.5 µl	**1
RNAi duplex (20 µM stock)	5 µl	2 µl	1 µl	0.4 µl	0.2 µl	0.05 µl	**2
Opti-MEM® I Medium for siRNA dilution	200 µl	100 µl	50 µl	20 µl	10 µl	2.5 µl	
Final siRNA concentration (nM)	10 nM	10 nM	10 nM	10 nM	10 nM	10 nM	

**1: Other medium without serum/antibiotic can be used to replace Opti-MEM.

**2: The final concentration of siRNA is 10 nM in this table. The final concentration can be reduced to 1 nM without reduction in knockdown efficacy for most of the siRNA we have tested. A titration test (for example, 1, 5, 10 nM) is recommended for new siRNA. The minimal amount with good knockdown efficiency is recommended.

Modified from: https://www.thermofisher.com/no/en/home/references/protocols/cell-culture/transfection-protocol/rnaimax-forward-transfections-lipofectamine.html and https://tools.thermofisher.com/content/sfs/manuals/Lipofectamine_RNAiMAX_Reag_protocol.pdf

Edited by Yang Jin 17/11/2017