Transfecting Stealth™ RNAi or siRNA into HUVEC Cells Using Lipofectamine™ RNAiMAX

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Introduction
Lipofectamine™ RNAiMAX Reagent is a proprietary formulation specifically developed for highly efficient delivery of Stealth™ RNAi or short interfering RNA (siRNA) to mammalian cells for RNAi analysis. This reference provides a recommended procedure to transfect Stealth™ RNAi or siRNA into human HUVEC umbilical vein endothelial cells (ATCC, Cat. No. CRL-1730) using Lipofectamine™ RNAiMAX (Cat. Nos. 13778-075, 13778-150). Lipofectamine™ RNAiMAX has a broad range of activity, enabling achievement of maximal knockdown levels with a minimum of optimization required.

Important Guidelines for Transfection
Follow these important guidelines when transfecting Stealth™ RNAi or siRNA into HUVEC cells using Lipofectamine™ RNAiMAX:

- Only **Forward Transfection** (page 2) has been tested for transfecting HUVEC cells.
- To assess transfection efficiency, we recommend using a KIF11 Stealth™ Select RNAi, as described in **Assessing Transfection Efficiency** (page 3).
- We recommend using 10 nM RNAi duplex and indicated procedures. However, the efficacy of the RNAi sequence chosen, the transcription rate of the target gene, and the stability of the resulting protein influence the degree of target gene knockdown observed. You may need to adjust the RNAi concentration used (1-50 nM can be used) and assay time (up to 72 hours) to establish optimal knockdown of your target gene.
- We recommend Opti-MEM® I Reduced Serum Medium (Cat. No. 31985-062) to dilute RNAi duplexes and Lipofectamine™ RNAiMAX before complexing.
- **Do not** add antibiotics to media during transfection as this causes cell death.
- Test serum-free media for compatibility with Lipofectamine™ RNAiMAX.
- 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).
Materials Needed
Have the following reagents on hand before beginning:

- HUVEC cells maintained in Ham’s Kaighn’s Modification F12 (F12K) (Cat. No. 21127-022) supplemented with 2mM L-Glutamine (Cat. No. 25030-081), 0.1mg/ml Heparin sodium salt from porcine intestinal mucosa (Sigma, Cat. No. H3393), 0.05mg/ml ECGS (Endothelial Cell Growth Supplement; BD Cat. No. 354006), 10% FBS (Cat. No. 16000-044). This medium should be prepared fresh every 2 weeks.
  
  Note: Use low-passage cells; make sure that cells are healthy and greater than 90% viable before transfection.

- Stealth™ RNAi (or siRNA) of interest

- Lipofectamine™ RNAiMAX Reagent (store at +4°C until use)

- Opti-MEM® I Reduced Serum Medium

- Appropriate tissue culture plates and supplies

Forward Transfection
Use this procedure to forward transfect Stealth™ RNAi or siRNA into HUVEC 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 35,000 cells in 500 µl of growth medium without antibiotics. The cell density should 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.
     
     Note: If the volume of your RNAi duplex solution is too small to dispense accurately (less than 1 µl), and you cannot pool dilutions, predilute your RNAi duplex 10-fold in 1X RNA Annealing/Dilution Buffer (or dilution buffer recommended by your RNAi duplex manufacturer), and dispense the proper higher amount (should be at least 1 µl per well). For example, to get 6 pmol of RNAi duplex from a 20 µM RNAi duplex stock solution, dilute your RNAi duplex 10-fold to a concentration of 2 µM, and dispense 3 µl.
   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, but this is not required.

Assessing Transfection Efficiency
To qualitatively assess transfection efficiency, we recommend using a KIF11 Stealth™ Select RNAi (available through www.invitrogen.com/rnaiexpress; for human cells, oligo HSS105842 is a good choice). Adherent cells in which KIF11/Eg5 is knocked down exhibit a “rounded-up” phenotype after 24 hours due to a mitotic arrest (Weil, D. et al., Biotechniques (2002), 33: 1244-1248); slow growing cells may take up to 72 hours to display the rounded phenotype. Alternatively, growth inhibition can be assayed after 48-72 hours.

Note: The BLOCK-it™ Fluorescent Oligo (Cat. No. 2013) is optimized for use with Lipofectamine™ 2000, and is not recommended for use with Lipofectamine™ RNAiMAX.
Acceptable Range for Maximal Activity

Due to the broad range of maximal activity exhibited by Lipofectamine™ RNAiMAX, a range of cell densities and volumes of Lipofectamine™ RNAiMAX can be used for transfection. For transfecting HUVEC cells in 24-well format, 0.5-1.25 µl Lipofectamine™ RNAiMAX and 30,000 – 40,000 cells per well is suitable. For extended time course experiments (72 hours), consider using the lower cell number; for short-term experiments (24 hours), consider the higher cell number.

The final concentration of RNAi duplex can be varied between 1-50 nM. A concentration of 10 nM RNAi duplex is suitable to knockdown many target genes. However, the optimal concentration of RNAi duplex will vary depending on the efficacy of the duplex, and should be determined empirically.

Recommended Reagent Amounts and Volumes

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

**Note:** 20 µM Stealth™ RNAi or siRNA = 20 pmol/µl.

<table>
<thead>
<tr>
<th>Culture vessel</th>
<th>Relative surface area</th>
<th>Volume of plating medium</th>
<th>Cells plated per well</th>
<th>Dilution medium</th>
<th>RNAi duplex amount</th>
<th>Final RNAi duplex conc.</th>
<th>Lipofectamine™ RNAiMAX²</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-well</td>
<td>0.2</td>
<td>100 µl</td>
<td>7,500</td>
<td>5,000-10,000</td>
<td>20</td>
<td>2 x 10</td>
<td>1.2</td>
</tr>
<tr>
<td>48-well</td>
<td>0.4</td>
<td>200 µl</td>
<td>15,000</td>
<td>10,000-20,000</td>
<td>40</td>
<td>2 x 20</td>
<td>2.4</td>
</tr>
<tr>
<td>24-well</td>
<td>1</td>
<td>500 µl</td>
<td>35,000</td>
<td>30,000-40,000</td>
<td>100</td>
<td>2 x 50</td>
<td>6</td>
</tr>
<tr>
<td>6-well</td>
<td>5</td>
<td>2.5 ml</td>
<td>100,000</td>
<td>90,000-120,000</td>
<td>500</td>
<td>2 x 250</td>
<td>30</td>
</tr>
</tbody>
</table>

¹Surface areas may vary depending on the manufacturer.
²If the volume of Lipofectamine™ RNAiMAX is too small to dispense accurately, and you cannot pool dilutions, predilute Lipofectamine™ RNAiMAX 10-fold in Opti-MEM® I Reduced Serum Medium, and dispense a 10-fold higher amount (should be at least 1.0 µl per well). Discard any unused diluted Lipofectamine™ RNAiMAX.

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