Transfecting Stealth™ RNAi or siRNA into HUVEC Cells Using Oligofectamine™

Introduction
Oligofectamine™ Reagent is a proprietary formulation that facilitates highly efficient delivery of Stealth™ RNAi or short interfering RNA (siRNA) to mammalian cells for RNAi analysis. This reference provides general guidelines and an optimized procedure to transfect Stealth™ RNAi (or siRNA) into human HUVEC umbilical vein endothelial cells (Cambrex BioScience, Cat. No. CC-2517) using Oligofectamine™ Reagent (Cat. No. 12252-011).

Important Guidelines for Transfection
Follow these important guidelines when transfecting Stealth™ RNA (or siRNA) into HUVEC cells using Oligofectamine™:
1. Use 300 nM Stealth™ RNAi (or siRNA) complexed with a 1:125 final dilution of Oligofectamine™ for transfection. To increase accuracy and reduce assay variability, we recommend performing transfection in triplicate for each sample condition.
2. Transfect HUVEC cells at 85-95% confluence.
3. Do not add antibiotics to the medium during transfection as this reduces transfection efficiency and causes cell death.
4. Use Opti-MEM® I Reduced Serum Medium (Cat. No. 31985-062) to dilute Oligofectamine™ and Stealth™ RNAi (or siRNA) prior to complex formation.

Materials Needed
Have the following reagents on hand before beginning:
- HUVEC cells maintained in Endothelial Cell Basal Medium (EBM®; Cambrex BioScience, Cat. No. CC-3121) supplemented with EGM SingleQuots (Cambrex BioScience, Cat. No. CC-4133)
  Note: Use low-passage cells (passage 4 or lower); make sure that cells are healthy and greater than 90% viable before transfection.
- Stealth™ RNAi (or siRNA) of interest (20 µM in annealing buffer)
- Oligofectamine™ Reagent (store at +4°C until use)
- Opti-MEM® I Reduced Serum Medium (pre-warm to 37°C before use)
- EGM (i.e. HUVEC culture medium) containing 3X the normal concentration of serum (3X concentration = 6% FBS)
- Appropriate tissue culture plates and supplies

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**Transfection Procedure**

Use this procedure to transfect Stealth™ RNAi (or siRNA) into HUVEC cells using Oligofectamine™ in a 24-well format. For other formats, see the table in **Recommended Reagent Amounts and Volumes** for the appropriate reagent amounts to add.

**Tip:** To reduce well-to-well variability when transfecting multiple replicates (e.g. triplicates), proportionally scale up the reagent volumes to form complexes (Step 2), then aliquot an equal volume of complexes into each well.

1. One day before transfection, plate 6 x 10^4 HUVEC cells in 500 µl of growth medium without antibiotics per well. Cells should be 85-95% confluent at the time of transfection.

2. For each transfection sample, prepare Stealth™ RNAi-Oligofectamine™ complexes as follows:
   a. Dilute 75 pmol of Stealth™ RNAi (i.e. 3.75 µl of 20 µM Stealth™ RNAi) in 36.25 µl of Opti-MEM® I Reduced Serum Medium. Mix gently.
   b. Mix Oligofectamine™ gently before use, then dilute 2 µl in 8 µl of Opti-MEM® I Reduced Serum Medium. Mix gently and incubate for 5 minutes at room temperature.
   c. After the 15-minute incubation, combine the diluted Stealth™ RNAi and the diluted Oligofectamine™ (total volume = 50 µl). Mix gently and incubate for 15 minutes at room temperature to allow complexes to form (solution may appear cloudy).

3. While complexes are forming, remove the growth medium from the cells and wash once with 500 µl of EGM containing 3X the normal concentration of serum (without antibiotics) without removing the transfection mixture.

4. Add the 50 µl of Stealth™ RNAi-Oligofectamine™ complexes (Step 2c) gently, and add to the cells (total volume = 250 µl). Mix gently by rocking the plate back and forth.

5. Incubate the cells at 37°C in a humidified CO₂ incubator for 4 hours.

6. Add 125 µl of EGM containing 3X the normal concentration of serum (without antibiotics) without removing the transfection mixture.

7. Incubate the cells at 37°C in a humidified CO₂ incubator for 16-24 hours as appropriate until you are ready to assay for gene knockdown.

**Recommended Reagent Amounts and Volumes**

To transfect HUVEC cells in different tissue culture formats, vary the amounts of Stealth™ RNAi (or siRNA), Oligofectamine™, cells, and medium used in proportion to the relative surface area, as shown in the table. **Note:** 20 µM Stealth™ RNAi or siRNA = 20 pmol/µl.

<table>
<thead>
<tr>
<th>Culture vessel</th>
<th>Relative surface area (vs. 24-well)</th>
<th>Cells plated per well</th>
<th>Stealth™ RNAi (pmol) &amp; dilution vol. (µl)</th>
<th>Oligofectamine™ (µl) &amp; final dilution vol. (µl)</th>
<th>Plating medium vol.</th>
<th>Total vol. per well</th>
<th>Added vol. medium with 3X serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>48-well</td>
<td>0.4</td>
<td>3 x 10⁴</td>
<td>30 pmol to 20 µl</td>
<td>1 µl to 5 µl</td>
<td>100 µl</td>
<td>125 µl</td>
<td>75 µl</td>
</tr>
<tr>
<td>24-well</td>
<td>1</td>
<td>6 x 10⁴</td>
<td>75 pmol to 40 µl</td>
<td>2 µl to 10 µl</td>
<td>200 µl</td>
<td>250 µl</td>
<td>125 µl</td>
</tr>
<tr>
<td>6-well</td>
<td>5</td>
<td>3 x 10⁵</td>
<td>375 pmol to 175 µl</td>
<td>8 µl to 25 µl</td>
<td>800 µl</td>
<td>1 ml</td>
<td>500 µl</td>
</tr>
</tbody>
</table>

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