

# Transfecting Stealth™ RNAi or siRNA into HMVEC-L Cells Using Lipofectamine™ 2000

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## Introduction

Lipofectamine™ 2000 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 HMVEC-L lung microvascular endothelial cells (Cambrex BioScience, Cat. No. CC-2527) using Lipofectamine™ 2000 (Cat. No. 11668-027).

**Note:** While transfection conditions have been optimized to allow highly efficient delivery of Stealth™ RNAi into HMVEC-L cells, other factors related to the target gene of interest including the transcription rate of the target gene, the stability of the resulting protein, and efficacy of the Stealth™ RNAi sequence chosen can influence the degree of target gene knockdown observed. Take these factors into consideration when designing your RNAi experiment.

## Important Guidelines for Transfection

Follow these important guidelines when transfecting Stealth™ RNA (or siRNA) into HMVEC-L cells using Lipofectamine™ 2000:

1. Use 200 nM Stealth™ RNAi (or siRNA) complexed with 2 µg/ml Lipofectamine™ 2000 (stock solution is 1 mg/ml) for transfection. To increase accuracy and reduce assay variability, we recommend performing transfection **in triplicate** for each sample condition.
2. Transfect HMVEC-L cells at 90% 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 Lipofectamine™ 2000 and Stealth™ RNAi (or siRNA) prior to complex formation.

## Materials Needed

Have the following reagents on hand before beginning:

- HMVEC-L cells maintained in Endothelial Cell Basal Medium 2 (EBM®-2; Cambrex BioScience, Cat. No. CC-3156) supplemented with EGM-2-MV SingleQuots (Cambrex BioScience, Cat. No. CC-4147)  
**Note:** Use low-passage cells (passage 6 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)
- Lipofectamine™ 2000 Reagent (store at +4°C until use)
- Opti-MEM® I Reduced Serum Medium (pre-warm to 37°C before use)
- Appropriate tissue culture plates and supplies

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## Transfection Procedure

Use this procedure to transfect Stealth™ RNAi (or siRNA) into HMVEC-L cells using Lipofectamine™ 2000 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.

- One day before transfection, plate  $6 \times 10^4$  HMVEC-L cells in 400  $\mu$ l of growth medium without antibiotics per well. Cells should be 90% confluent at the time of transfection.
- For each transfection sample**, prepare Stealth™ RNAi-Lipofectamine™ 2000 complexes as follows:
  - Dilute 100 pmol of Stealth™ RNAi (i.e. 5  $\mu$ l of 20  $\mu$ M Stealth™ RNAi) in 50  $\mu$ l of Opti-MEM® I Reduced Serum Medium. Mix gently.
  - Mix Lipofectamine™ 2000 gently before use, then dilute 1  $\mu$ l in 50  $\mu$ l of Opti-MEM® I Reduced Serum Medium. Mix gently and incubate for 15 minutes at room temperature.
  - After the 15-minute incubation, combine the diluted Stealth™ RNAi and the diluted Lipofectamine™ 2000 (total volume = 106  $\mu$ l). Mix gently and incubate for 15 minutes at room temperature to allow complexes to form (solution may appear cloudy).
- Add the 106  $\mu$ l of Stealth™ RNAi-Lipofectamine™ 2000 complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth.
- Incubate the cells at 37°C in a humidified CO<sub>2</sub> incubator for 16-24 hours as appropriate until you are ready to assay for gene knockdown. It is not necessary to remove the complexes or change the medium; however, growth medium may be replaced after 4-6 hours without loss of transfection activity.

## Recommended Reagent Amounts and Volumes

To transfect HMVEC-L cells in different tissue culture formats, vary the amounts of Stealth™ RNAi (or siRNA), Lipofectamine™ 2000, cells, and medium used in proportion to the relative surface area, as shown in the table. **Note:** 20  $\mu$ M Stealth™ RNAi or siRNA = 20 pmol/ $\mu$ l.

Culture vessel	Relative surface area (vs. 24-well)	Cells plated per well	Volume of plating medium	Stealth™ RNAi (pmol) in media volume ( $\mu$ l)	Lipofectamine™ 2000 ( $\mu$ l) in media volume ( $\mu$ l)
48-well	0.4	$3 \times 10^4$	200 $\mu$ l	40 pmol in 25 $\mu$ l	0.4 $\mu$ l in 25 $\mu$ l
24-well	1	$6 \times 10^4$	400 $\mu$ l	100 pmol in 50 $\mu$ l	1 $\mu$ l in 50 $\mu$ l
6-well	5	$3 \times 10^5$	2 ml	500 pmol in 250 $\mu$ l	5 $\mu$ l in 250 $\mu$ l

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