Transfecting Stealth™ RNAi or siRNA into SK-N-SH Cells Using Lipofectamine™ RNAiMAX

Terms and Conditions of Use

Invitrogen Corporation ("Invitrogen") grants to you, its customer, a non-exclusive, non-transferable license to access the Invitrogen RNAi transfection protocol applicable to Invitrogen products, as set forth below (the "Protocol"). You acknowledge that the Protocol is protected by copyright. All rights not specifically granted to you are expressly reserved to Invitrogen. You may use the Protocol for personal, educational, or scientific research or professional use, but in no case for a fee. You may not remove, obscure or modify any copyright or proprietary notices, author attribution or any disclaimer contained in the Protocol.

INVITROGEN PROVIDES THE PROTOCOL "AS IS", WITHOUT WARRANTIES OF ANY KIND, EITHER EXPRESS OR IMPLIED, INCLUDING, BUT NOT LIMITED TO, WARRANTIES OF TITLE, OR IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE OR NON-INFRINGEMENT; YOUR USE OF THE PROTOCOL IS AT YOUR OWN RISK; NEITHER INVITROGEN NOR ITS AFFILIATES, EMPLOYEES, OFFICERS OR DIRECTORS SHALL BE LIABLE TO YOU OR ANY THIRD PARTY FOR ANY DIRECT, INDIRECT, INCIDENTAL, SPECIAL, CONSEQUENTIAL OR PUNITIVE DAMAGES ARISING OUT OF THIS LICENSE OR ANY USE OF THE PROTOCOL. Some jurisdictions do not allow the exclusion or limitation of liability for certain damages, so the above limitation may not apply to you to the extent prohibited by such local laws; if so, then Invitrogen's liability for damages hereunder shall not exceed an amount equal to the amounts paid by you hereunder, or one hundred dollars ($100.00), whichever is less.

Confidentiality

You acknowledge the Protocol is Invitrogen's confidential information and constitutes Invitrogen's proprietary and valuable trade secrets. You agree to take reasonable care to protect the Protocol from disclosure to third parties, except as may be expressly authorized by Invitrogen.

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 SK-N-SH neuroblastoma cells (ATCC, Cat. No. HTB-11) 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 SK-N-SH cells using Lipofectamine™ RNAiMAX:

- Both **Reverse Transfection** and **Forward Transfection** protocols (page 2) can be used for transfecting SK-N-SH 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:

- SK-N-SH cells maintained in MEM with Earle's salts and L-glutamine (Cat. No. 11095-080) supplemented with 10% fetal bovine serum (Cat. No. 26140-079), 0.1 mM MEM non-essential amino acids (Cat. No. 11140-050), 1 mM sodium pyruvate (Cat. No. 11360-070), and penicillin/streptomycin (Cat. No. 15070-063)
  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

Reverse Transfection

Use this procedure to reverse transfect Stealth™ RNAi or siRNA into SK-N-SH 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.
      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 a 10-fold 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 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 SK-N-SH cells in complete growth medium **without antibiotics** so that 500 µl contains 30,000 cells (cell density should be 30-50% confluent 24 hours after plating).
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.

Forward Transfection

Use this procedure to forward transfect Stealth™ RNAi or siRNA into SK-N-SH 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 30,000 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 CO_2 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 SK-N-SH cells in 24-well format, 0.75-1.5 µl Lipofectamine™ RNAiMAX and 20,000 – 50,000 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 SK-N-SH 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.

Culture vessel	Rel. surf. area[1]	Volume of plating medium	Cells plated per well		Dilution medium		RNAi duplex amount		Final RNAi duplex conc.		Lipofectamine™ RNAiMAX[2]	
			Start point	Acceptable Range	Reverse transf. (µl)	Forward transf. (µl)	Start point (pmol)	Acc. Range (pmol)	Start point (nM)	Acc. Range (nM)	Start point (µl)	Acc. Range (µl)
96-well	0.2	100 µl	7,500	5,000-10,000	20	2 x 10	1.2	0.12-6	10	1-50	0.2	0.15-0.3
48-well	0.4	200 µl	15,000	10,000-20,000	40	2 x 20	2.4	0.24-12	10	1-50	0.4	0.3-0.6
24-well	1	500 µl	30,000	20,000-50,000	100	2 x 50	6	0.6-30	10	1-50	1	0.75-1.5
6-well	5	2.5 ml	150,000	100,000-250,000	500	2 x 250	30	3-150	10	1-50	5	3.75-7.5

[1] Surface areas may vary depending on the manufacturer.

[2] If 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.

Limited Use Label License No. 5: Invitrogen Technology

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of patents owned or controlled by Invitrogen Corporation which cover this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500. Email: outlicensing@invitrogen.com

Limited Use Label License No. 27: RNA Transfection

Use of this product in conjunction with methods for the introduction of RNA molecules into cells may require licenses to one or more patents or patent applications. Users of these products should determine if any licenses are required.

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

©2006 Invitrogen Corporation. All rights reserved.