Product Number(s): NI35150, NI35750

i-Fect™ siRNA Transfection Reagent

Instruction Manual

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This product is manufactured for Neuromics Antibodies by Gene Therapy Systems

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OVERVIEW
Kit Contents

Catalogue Number	Number of Tubes	Description	Size or Aliquot
NI35150	1 vial	Hydrated i-Fect™ lipid	750 µl
	1 vial	siRNA diluent	4 ml
NI35750	5 vials	Hydrated i-Fect™ lipid	750 µl
	5 vials	siRNA diluent	4 ml

Each i-Fect siRNA Transfection Reagent tube (750 µl) contains sufficient martieral for 200 transfections based on transfecting 200 ng of siRNA.

Shipping and Storage

The i-Fect™ siRNA Transfection Reagent is shipped at room temperature. For maximum stability, store all reagents at 4ºC upon receipt. If stored properly, all components are stable for 12 months.

Product Support

Telephone: 612-801-1007 OR 866-350-1500 (US toll free)
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Introduction

RNA interference (RNAi) is emerging as an effective tool for inhibiting gene expression in a variety of organisms (*e.g.* plants, insects, and nematodes) and cultured mammalian cell lines. It is characterized by targeted mRNA degradation after introduction of sequence-specific double stranded RNA (dsRNA) into cells. Although cellular uptake of long dsRNA by organisms such as *C. elegans* and *Drosophila* has proven to be an effective method to induce RNAi, it tends to result in nonspecific gene suppression in vetebrate cells due in part to interferon response. Recently, it has been discovered that short (less than 30 nucleotides) dsRNA, referred to as small interfering RNAs (siRNA), can cause gene-specific silencing in mammalian cells (1,2,3). In addition, the RNAi effect caused by siRNA can be detectable even after many cell divisions. These properties make siRNA transfection a useful tool for gene silencing in mammalian cells.

i-Fect™ siRNA Transfection Reagent is developed to allow researchers to fully take advantage of the powerful RNAi technique. It is a cationic lipid based reagent that has been extensively screened in many mammalian cell lines in order to achieve:

- Efficient delivery of siRNA
- Functional gene silencing post siRNA delivery
- Compatibility with diverse growth conditions (with and without serum)
- Lowest cytotoxicity

When compared to other commercially available transfection reagents, i-Fect™ consistently offers superior transfection efficiencies and more effective gene silencing.

METHODS AND PROCEDURES

1. Transfection of Adherent Cells

1.1. The day before transfection, plate cells so that they will be 50-70% confluent on the day of transfection.

1.2. Prepare the i-Fect™ reagent by diluting in serum free medium according to Table 1 below.

Table 1: i-Fect™ Dilutions for Adherent Cells

Tissue culture plate or dish type	i-Fect™ Reagent (µl) + Serum Free Medium (µl) per well
96 wells	1.0 + 25
48 wells	1.75 + 25
24 wells	3.5 + 25
6 wells	5.0 + 25

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1.3. Prepare the siRNA solution by first mixing siRNA Diluent and serum free medium (SFM) according to Table 2 below. Use the Diluent/SFM mix to dilute the recommended amount of siRNA in Table 2. Mix well by pipetting up and down several times. Incubate at Room Temperature for 5 minutes.

NOTE *Avoid vortexing the siRNA/Diluent mix.*

Table 2: siRNA Dilutions for Adherent Cells

Tissue Culture plate type	Recommended amount of siRNA to use (ng) per well	siRNA Diluent (µl)+ Serum Free Medium (µl) per well	Final transfection volume (µl)
96 wells	50	2.5 + 15	100
48 wells	100	5.0 + 15	200
24 wells	200	10.0 + 15	500
6 wells	1,000	25.0 + 15	1,000

1.4. Add the RNA solution from Step 1.3 to the diluted i-Fect solution in Step 1.2. Incubate at Room Temperature for 5 minutes to allow the siRNA/lipid complexes to form.

NOTE *You can incubate the siRNA/i-Fect™ mix for longer than 5 minutes, but make sure not to exceed 30 minutes in order to maintain maximum siRNA transfection efficiency.*

1.5. Add the siRNA/i-Fect™ mix to cells growing in serum-containing medium. Incubate at 37°C for 24 hours. See Table 2 for transfection volume.

TIPS *For some cell lines like HeLa, MDCK, and CHO-K1, transfection efficiencies may be higher if serum is omitted in the medium during the first 4 hours of transfection. After this step, add one volume of medium containing 20% serum, then proceed to step 1.6.*

1.6. Add fresh tissue culture medium to growing cells as needed. Most RNA interference can be detected within 24 to 72 hours post transfection.

2. Transfection of Suspension Cells

2.1. The day before transfection, split the cells as necessary to optimize their health and achieve loggrowth by transfection time.

2.2. Prepare the i-Fect™ reagent by diluting in serum free medium according to Table 3 below.

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Table 3: i-Fect™ Dilutions for Suspension Cells

Tissue culture plate type	i-Fect™ Reagent (µl) + Serum Free Medium (µl) per well
96 wells	1.0 + 25
48 wells	1.75 + 25
24 wells	3.5 +25
6 wells	5.0 + 25

2.3. Prepare the siRNA solution by first mixing siRNA Diluent and serum free medium (SFM) according to Table 4 below. Use the Diluent/SFM mix to dilute the recommended amount of siRNA in Table 4. Mix well by pipetting up and down several times. Incubate at Room Temperature for 5 minutes.

NOTE *Avoid vortexing the siRNA Diluent mix.*

Table 4: siRNA Dilutions for Suspension Cells

Tissue Culture plate or dish type	Recommended amount of siRNA to use (ng) per well	siRNA Diluent (µl)+ serum free medium (µl) per well
96 wells	50	2.5 + 15
48 wells	100	5.0 + 15
24 wells	200	10.0 + 15
6 wells	1,000	25.0 + 15

2.4. Add the RNA solution from Step 2.3 to the diluted i-Fect™ solution in Step 2.2. Incubate at Room Temperature for 5 minutes to allow the siRNA/lipid complexes to form.

NOTE *You can incubate the siRNA/i-Fect mix for longer than 5 minutes, but make sure not to exceed 30 minutes in order to maintain maximum siRNA transfection efficiency.*

2.5. While the siRNA/i-Fect mix is incubating, spin down the cells from Step 2.1, remove the growth medium, and then resuspend the cells in the appropriate growth medium (serum-free or serum-containing) to achieve a final density of 10^6 cells/ml.

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2.6. Transfer resuspended cells to culture plates according to Table 5 below.

Table 5: Volume and Number of Cells to Transfer into Culture Dishes.

Tissue culture plate or dish type	Volume of resuspended cells to transfer to each well (ml)	Number of cells transferred to each well (approximate).
96 wells	0.1	1×10^5
48 wells	0.2	2×10^5
24 wells	0.5	5×10^5
6 wells	1.0	2×10^6

2.7. Add the siRNA/i-Fect™ mix to resuspended cells in Table 5 above. Gently mix the cells by pipetting up and down several times; this step is important to avoid cell clumping. Incubate at 37°C for 24 hours.

2.8. Add fresh tissue culture medium to growing cells as needed. Most RNA interference can be detected within 24 to 72 hours post transfection.

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APPENDIX

Transfection Optimization Guidelines

A. Adherent Cells

Although i-Fect™ consistently delivers high transfection efficiencies in a wide range of cell types, to obtain maximum efficiency in particular cell lines some optimization may be needed. The two critical variables are the i-Fect™/siRNA ratio and the siRNA quantity. To optimize these two variables:

1. Determine the best i-Fect™/siRNA ratio by using 0.5 - 7 µl of reagent for each 100 ng of siRNA. Use a low siRNA quantity to optimize this parameter.

2. Once the optimal ratio has been established, vary the siRNA quantity over the suggested range. At this point, cell number can also be optimized.

B. Suspension Cells

For suspension cells the optimization procedure is the same as for adherent cells except that the iFect™/siRNA ratio is higher.

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Troubleshooting Guide

Problem	Possible Causes	Recommended Solutions
Low transfection efficiency	Suboptimal i-Fect /siRNA ratio.	Optimize the i-Fect/siRNA ratio by using 0.5-7 µl of i-Fect for each 100 ng of siRNA. Use a low siRNA quantity to optimize this parameter.
	Suboptimal siRNA concentration.	After establishing the optimal i-Fect/siRNA ratio, vary the siRNA quantity over the ranges suggested in the Methods and Procedures section.
	Poor siRNA quality	Use RNase-free handling procedures and plasticware. siRNA should be gel-purified. Check for degradation of siRNA on acrylamide gels.
	Denatured siRNA	Use recommended buffer (100 mM NaCl, 50 mM Tris, pH 7.5 in RNase-free water) to dilute siRNA. Do not use water as it can denature the siRNA.
	Cells have been in continous passage for > 2 months	Thaw out a fresh aliquot of cells and passage once (or more) before transfecting. Avoid using cells that have been in culture or have been passaged for excessive periods of time.
	Suboptimal cell density.	Use cells that are 50-70% cconfluent on the day of transfection. Optimal cell density may vary depending on cell type.
	Improper storage.	i-Fect reagent is very stable but long exposure to elevated temperatures and/or excessive freeze/thaw cycles may cause degradation of the reagent. Store i-Fect reagent at 4° C.
	Wrong medium.	Be sure to use serum-free medium when forming the i-Fect/siRNA complex.
	Cell line is difficult to transfect.	Optimize i-Fect/siRNA ratio and siRNA amount as indicated in Appendix.
	i-Fect/ siRNA complexes not freshly prepared.	i-Fect/siRNA complexes should be freshly prepared. If complexes have been prepared and stored for longer than 45 minutes, aggregation may occur.
	Suboptimal i-Fect /siRNA ratio used.	Too much i-Fect or too much siRNA could cause aggregation; Adjust the ratio as outlined above.
Aggregation	Excess i-Fect used.	Decrease the amount of i-Fect reagent.
Cytotoxicity	Unhealthy cells.	• Check cells for contamination. • Thaw a new batch of cells. • Cells are too confluent or cell density too low. • Check culture medium (pH, kind used, last time changed). - Check materials used for proper function (culture plates, incubator temperatures, etc.).
	i-Fect concentration too high	Reduce i-Fect concentration in 20-30% increments.

References:

1. Elbashir, S.M. *et al.* (2001) *Nature* **411:** 494-498.
2. Caplen, N.J. *et al.* (2001) *Proc Natl Acad Sci USA* **98:** 9742-9747.
3. Sharp, P.A. (2001) *Genes and Development* **15:** 485-490.

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