Product Number(s):

PL30001/PL30004	**1 plate/4plates**	**Standard**
PL30002/PL30005	**1 plate/4 plates**	**High**
PL30003/PL30006	**1 plate/4 plate**	**Low**

i-Fect™ Hi-Put 96

96-well high-throughput system for efficient delivery of siRNA

Instruction Manual

Neuromics
5325 West 74th Street, Suite 8
Edina, MN 55438
Phone: 952-374-6161
Fax: 612-677-3976
Email: pshuster@neuromics1.com
Website: www.neuromics.com

Purchaser Notification

Limited License

The purchase price paid for the i-Fect™ Hi-Put 96 Transfection Reagent kit by end users grants them a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in the Kit Contents section). This kit is intended **for internal research only** by the purchaser. Such use is limited to the transfection of nucleic acid into neuronal cells as described in the product manual. Furthermore, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of Neuromics.

Purchasers may terminate this License at any time by returning all i-Fect Hi-Put 96 Reagent material and documentation to Neuromics, or by destroying all i-Fect Hi-Put 96 Reagent kit components.

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Product Use Limitations

The i-Fect Hi-Put 96 Reagent and all of its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the handling of the kit components by following appropriate research lab practices.

For more information or any comments on the terms and conditions of this License, please contact:

Neuromics
5325 West 74th Street, Suite 8
Edina, MN 55438
Email: **phuster@neuromics.com**

This product is manufactured for Neuromics by Genlantis, a division of Gene Therapy Systems

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phone 952-374-6161 • fax 612-677-3976 • e-mail pshuster@neuromics1.com

Overview

Kit Contents

Catalog#	Description	Contents
PL30001	i-Fect Hi-Put 96 siRNA Transfection Reagent Titration Plate	1 x 96-well plate containing 11.7 µl to 0.15 µl of i-Fect Reagent per well. 1 x 0.5 ml siRNA Diluent.
PL30004	i-Fect Hi-Put 96 siRNA Transfection Reagent Titration Plate	4 x 96-well plates containing 11.7 µl to 0.15 µl of i-Fect Reagent per well. 4 x 0.5 ml siRNA Diluent.
PL30002	i-Fect Hi-Put 96 siRNA Transfection Reagent Titration Plate (High)	1 x 96-well plate containing 3.5 µl of i-Fect Reagent per well. 1 x 0.5 ml siRNA Diluent.
PL30005	i-Fect Hi-Put 96 siRNA Transfection Reagent Titration Plate (High)	4 x 96-well plates containing 3.5 µl of i-Fect Reagent per well. 4 x 0.5 ml siRNA Diluent.
PL30003	i-Fect Hi-Put 96 siRNA Transfection Reagent Titration Plate (Low)	1 x 96-well plate containing 0.7 µl of i-Fect Reagent per well. 1 x 0.5 ml siRNA Diluent.
PL30003	i-Fect Hi-Put 96 siRNA Transfection Reagent Titration Plate (Low)	4 x 96-well plates containing 0.7 µl of i-Fect Reagent per well. 4 x 0.5 ml siRNA Diluent.

Shipping and Storage

The i-Fect Hi-Put 96 siRNA Transfection Reagent Plates are shipped at room temperature. For maximum stability, store all reagents at 4^{o}C upon receipt. ***Keep plates sealed while in storage to prevent evaporation and/or contamination.*** If stored properly, all components are stable for 6 months.

Product Support

Telephone: 952-374-6161 or 866-350-1500 (US toll free)
Fax: 612-677-3976
E-mail: pshuster@neuromics1.com

Introduction

i-Fect™ Hi-Put siRNA Transfection Reagent Plates provide a convenient high-throughput format for optimizing transfection conditions and performing siRNA transfection in 96-well plates. All wells (except those in Column 12) contain a predetermined amount of the i-Fect siRNA Transfection Reagent, 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).
- Low cytotoxicity.

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

I-Fect Hi-Put 96siRNA Transfection Reagent Plates are provided in 3 related configurations:

1. The **i-Fect Hi-Put 96 siRNA Transfection Reagent Titration Plate** contains serially diluted transfection reagent across the columns of the plate. Each well contains 11.7 - 0.15 µl of lipid diluted in serum-free media to a final volume of 25 µl. This format allows for the determination of the optimum amount of i-Fect reagent needed for maximal siRNA delivery. Additionally, optimal siRNA quantity may be determined by the addition of decreasing amounts of siRNA down the rows of the plate.
2. The **i-Fect Hi-Put 96 siRNA Transfection Reagent Standard Plate (High)** contains the fixed amount of 3.5 µl of i-Fect siRNA Transfection Reagent + 21.5 µl of serum free medium in each well of the plate. This allows for rapid transfections in a 96-well format when a higher optimal volume of i-Fect has been established using the Titration Plate or from previous experiments or data.
3. The **i-Fect Hi-Put 96 siRNA Transfection Reagent Standard Plate (Low)** contains the fixed amount of 0.7 µl of i-fect siRNA Transfection Reagent + 24.3 µl of serum free medium in each well of the plate. This allows for rapid transfections in a 96-well format when a lower optimal volume of i-Fect has been established using the Titration Plate or from previous experiments or data.

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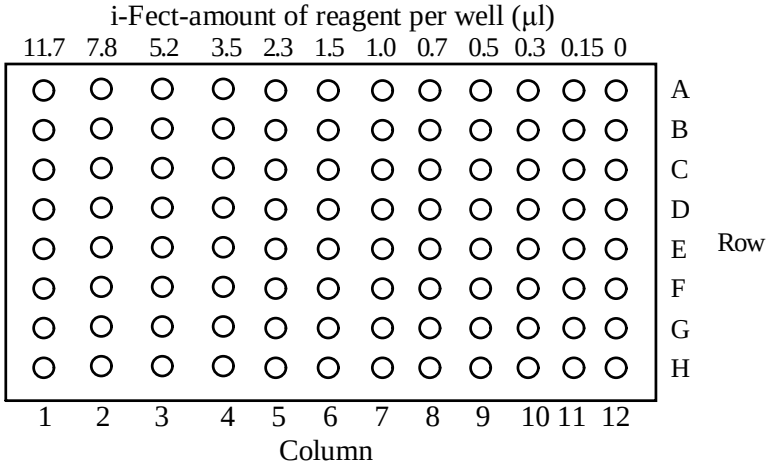
Method and Procedures

1. i-Fect 96 siRNA Transfection Reagent Titration Plate

1.1. The day before transfection split the cells so they are in good condition on the day of transfection.

1.2. Establish a range of siRNA amounts to dispense in each row of the Plate, *e.g.* 20 ng – 240 ng.

Table 1: Titration Plate Layout



1.3. Centrifuge the Titration Plate in a swinging platform rotor at 2,000 to 3,000 rpm (~1000 x g) for at least 1 minute so that the 25 µl of diluted i-Fect reagent will be collected at the bottom of each well. Peel away the adhesive seal by holding the plate firmly on a flat surface and pealing away the seal gently using the white tabs on either end.

1.4. If using adherent cells, detach cells from well surface by adding 5% trypsin/EDTA and incubating approximately 3 minutes. Inactivate the trypsin by adding an equal volume of 20% serum-containing medium to the well. If using suspension cells, no trypsinization step is necessary.

1.5. Spin down the cells and resuspend to the recommended number per well (see Table 2, p. 8) in a final volume of 40 µl of culture medium.
TIP *For most cell types, we recommend using 10% serum-containing media in step 1.5. However, for some cell lines like HeLa, MDCK, and CHO-K1, transfection efficiencies may be higher if serum is omitted from the medium at this step until 4 hours after the start of transfection. Subsequently, add one volume of medium containing 20% serum, then proceed to step 1.9.*

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Table 2: Recommend Seeding Numbers for Common Cell Types in a 96-Well Plate

Cell Type	Cells/Well	Cell Type	Cells/Well
B16-F0	10,000	HeLa	35,000
BHK-21	10,000	HepG2	25,000
CHO-K1	15,000	K562	75,000
COS-1	12,000	MDCK	8,000
COS-7	10,000	NIH-3T3	12,000
CV-1	12,000	PC-12	10,000
HEK-293	25,000		

1.6. Prepare your siRNA by mixing it with a combination of the siRNA Diluent (provided) and serum free medium (SFM) to make a total volume of 35 µl. First, mix the siRNA Diluent with SFM. Then use the Diluent/SFM mix to dilute your siRNA. Use 1 µl of siRNA Diluent per 20 ng of siRNA. Mix well by pipetting up and down several times. Incubate at Room Temperature for 5 minutes.

NOTE *1.We recommend you make a master mix of siRNA Diluent + SFM + your siRNA.*
2. Avoid vortexing the siRNA/Diluent mix.

1.7. Add the 35 µl of diluted siRNA solution from Step 1.6 above to the 25 µl of GeneSilencer™ solution contained in each well of the Incubation Plate. Incubate at Room Temperature for 5 min. to allow the siRNA/lipid complexes to form.

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

1.8. Add the 40 µl of cells to the siRNA/GeneSilencer® complexes in Titration Plate. The total transfection volume should now be 100 µl. Mix well by pipetting up and down several times. Incubate at 37°C for 24 hours.

NOTE *The morphology of adherent cells appears less healthy with this procedure than if the siRNA/GeneSilencer® complexes were added to seeded cells. However, we have found that transfection efficiency is actually enhanced when detaching the cells before transfection.*

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

2. i-Fect Hi-Put 96 siRNA Transfection Reagent Standard Plate (Both High and Low)

2.1. The day before transfection split the cells so they are in good condition on the day of transfection.

2.2. Centrifuge the Standard Plate in a swinging platform rotor at 2,000 to 3,000 rpm (~1000 x g) for at least 1 minute so that the 25 µl of diluted GeneSilencer® reagent will be collected at the bottom of each well. Peel away the adhesive seal by holding the plate firmly on a flat surface and pealing away the seal gently using the white tabs on either end.

2.3. If using adherent cells, detach from well surface by adding 5% trypsin/EDTA and incubating approximately 3 minutes. Inactivate the trypsin by adding an equal volume of 20% serumcontaining medium to the well. If using suspension cells, no trypsinization step is necessary.

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2.4. Spin down the cells and resuspend to the recommended number per well (see Table 2) in a final volume of 40 µl of culture medium.
TIP *For most cell types, we recommend using 10% serum-containing media in step 2.4. However, for some cell lines like HeLa, MDCK, and CHO-K1, transfection efficiencies may be higher if serum is omitted from the medium at this step until 4 hours after the start of transfection. Subsequently, add one volume of medium containing 20% serum, then proceed to step 2.9.*

2.5. Based on the optimum ratio of siRNA: i-Fect Reagent determined from the Titration Plate, calculate how much siRNA is needed per well based on 0.5 µl of i-Fect Reagent per well.

2.6 Prepare your siRNA by mixing it with a combination of the siRNA Diluent (provided) and serum free medium (SFM) to make a total volume of 35 µl. First, mix the siRNA Diluent with SFM. Then use the Diluent/SFM mix to dilute your siRNA. Use 1 µl of siRNA Diluent per 20 ng of siRNA. Mix well by pipetting up and down several times. Incubate at Room Temperature for 5 minutes.
NOTES *1.We recommend you make a master mix of siRNA Diluent + SFM + your siRNA*
2. Avoid vortexing the siRNA/Diluent mix.

2.7. Add the 35 µl of diluted siRNA solution from Step 2.6 above to the 25 µl of GeneSilencer® solution contained in each well of the Plate. Incubate at Room Temperature for 5 minutes to allow the siRNA/lipid complexes to form.
TIP *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.*

2.8. Add the 40 µl of cells to the siRNA/i-Fect complexes in Titration Plate. The total transfection volume should now be 100 µl. Mix well by pipetting up and down several times. Incubate at 37°C for 24 hours.
NOTE *The morphology of adherent cells appears less healthy with this procedure than if the siRNA/i-Fect complexes were added to seeded cells. However, we have found that transfection efficiency is actually enhanced when detaching the cells before transfection.*

2.9 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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Troubleshooting

Problem	Possible Causes	Recommended Solutions
Low transfection efficiency	Suboptimal siRNA concentration.	When establishing the optimal i-Fect/siRNA ratio using the Titration Plate, vary the siRNA quantity over the ranges suggested in Table 1.
	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. 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.
	Cells have been in continuous passage for more than 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% confluent 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 GeneSilencer™ 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 GeneSilencer®/siRNA ratio and siRNA amount by using the Titration Plate.
Aggregation	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. Test the ratios suggested in Table 1.
Cytotoxicity	i-Fect concentration too high relative to cell numbers.	Increase cell numbers in 10-20% increments.
	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.).

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