Product Number(s): NF30150, NF30750

n-Fect™ Transfection Reagent

Instruction Manual

DNA transfection kit for the Neuroscientist

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This product is manufactured for Neuromics Antibodies by Gene Therapy Systems

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OVERVIEW
Kit Contents

Each n-Fect Transfection Reagent kit (Cat. No. NF30150) contains sufficient material for 75 to 300 transfection reactions depending on the cell type. Each n-Fect™ Transfection Reagent kit (Cat. No. NF30750) contains sufficient material for 375 to 1500 transfection reactions depending on the cell type. Each reaction is for transfecting 2 µg of DNA.

Item	Description	Cat # NF30150	Cat # NF30750
n-Fect ™ Lipid	Dried n-Fect™ lipid film transfection reagent	1 vial	5 vials
Hydration Buffer	Transfection grade hydration buffer used to hydrate n-Fect™ dried lipid film before transfection	1 vial x 1.5 ml	5 vials x 1.5ml
DNA Diluent	Solution for diluting DNA for optimal transfection efficiency in neuronal cell lines	1 vial x 7.5 ml	5 vials x 7.5 ml

Shipping and Storage

The n-Fect™ Transfection Reagent kit is shipped at room temperature. For maximum stability store all reagents at 4°C upon receipt. All components are stable for at least one year if stored properly.

Product Support

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

n-Fect™ Transfection Reagent is a novel cationic lipid specially formulated for optimal transfection in neuronal cells, including primary neurons, differentiated post-mitotic neurons, neuronal cell lines, and glial cells. n-Fect™ Transfection Reagent is compatible with serum eliminates the need to change media following transfection. An included DNA Diluent is designed to facilitate DNA/lipid complex (lipoplex) formation and enhance the transformation efficiency in certain neuronal cells such as NT2 (not recommended for primary and differentiated neurons). Cell type specific protocols are developed for nFect™ Transfection Reagents to ensure optimal transfection results.

METHODS AND PROCEDURES

1. Transfection of Primary Neurons

1.1. Hydrate the n-Fect lipid vial at room temperature with 1.5 ml of the hydration buffer. Vortex for 30-60 seconds at top speed. Store the hydrated reagent at 4ºC and vortex briefly before use.

1.2. Dilute the hydrated n-Fect reagent with serum-free medium. Refer to Table 1 for the appropriate volume of serum-free medium.

Table 1: Volumes of Transfection Reagents

DNA (µg)	Serum Free Medium for DNA (µl)	n-Fect (µl)	Serum Free Medium for n-Fect (µl)
0.5	12.5	2.5	10
1	20	5	15
2	40	10	30
4	55	20	35
6	70	30	40
8	110	40	70

Although n-Fect has been optimized for specific cell culture conditions, optimization may be needed to achieve maximum transfection efficiency. The two critical variables are the ratio of n-Fect reagent to DNA and the quantity of DNA used. For optimization of the ratio of n-Fect reagent to DNA start by using 2.5 to 15 µl of reagent for each 1 µg of DNA. Use a fixed amount of DNA or vary the amount as suggested in the Appendix to optimize this ratio.

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1.3. Dilute the DNA with the serum free medium **(do not use the DNA Diluent for primary neurons)**. Refer to Table 1 for the appropriate volume of serum-free medium.

To obtain maximum efficiency in particular cells, some optimization may be needed. The two critical variables are the ratio of n-Fect reagent to DNA and the quantity of DNA used. For optimization of the DNA quantity used, maintain a fixed ratio of n-Fect reagent to DNA, and then vary the DNA quantity over a suggested range (see Table 2). See the Appendix for examples.

1.4. Add the DNA solution to the diluted n-Fect Transfection Reagent. Incubate at room temperature for 5 to 10 minutes to allow the n-Fect /DNA complexes to form.

Do not incubate DNA solution with the n-Fect Transfection Reagent for longer than 30 minutes

1.5. Add your complexes directly to the cells growing in serum-containing culture medium. Refer to Table 2 for suggested medium volumes.

Table 2: Medium Volumes and DNA Amount for Various Culture Dishes

Tissue Culture Dish	DNA (µg)	Medium Volume (ml)
96-well	0.1-0.5	0.2
24-well	0.5-3	0.5
12-well	1-4	1
6-well	2-6	1.5
60 mm	6-8	2.5
100 mm	8-12	5

1.6. Add fresh growth media as needed 24 hours post transfection. Depending on the cell type and promoter activity, the assay for the reporter gene can be performed 24 to72 hours following transfection.

For some cell types, the old media can be replaced with fresh media at this step.

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2. Transfection of Neuronal Cell Lines

2.1. Hydrate n-Fect lipid film at room temperature with 1.5 ml of the hydration buffer. Vortex for 3060 seconds at top speed. Store the hydrated reagent at 4ºC and vortex briefly before use.

2.2. Dilute the hydrated n-Fect reagent with serum-free medium. Refer to Table 3 for the appropriate volume of serum-free medium.

Table 3: Volumes of Transfection Reagents

DNA (µg)	DNA Diluent (µl)	n-Fect (µl)	Serum Free Medium for n-Fect (µl)
0.5	6.25	1.25	5
1	12.5	2.5	10
2	25	5	20
4	50	10	40
6	75	15	60
8	100	20	80

2.3. Dilute the DNA with the DNA Diluent and incubate 1 to 5 minutes at room temperature. Refer to Table 3 for the appropriate volume of DNA Diluent.

Do not incubate DNA with DNA Diluent for more than 5 min. Avoid vortexing the DNA Diluent.

Although n-Fect consistently delivers high transfection efficiencies, in order to obtain maximum efficiency in particular cell types, some optimization may be needed. The two critical variables are the ratio of n-Fect reagent to DNA and the quantity of DNA used. For optimization, first maintain a fixed ratio of n-Fect reagent to DNA, and then vary the DNA quantity over the suggested range. If necessary, optimize the ratio of n-Fect reagent to DNA by using 1.25 to 12.5

µl of reagent for each 1 µg of DNA. Use a low DNA quantity to optimize this ratio. Following this process, cell number can also be optimized. See the Appendix for examples.

2.4. Add the DNA solution to the diluted n-Fect Transfection Reagent. Incubate at room temperature for 5 to 10 minutes to allow the n-Fect /DNA complexes to form.

Do not incubate DNA solution with n-Fect Transfection Reagent for more than 30 minutes.

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2.5 Add your complexes directly to the cells growing in serum-containing culture medium. Refer to Table 4 for suggested cell numbers for specific tissue culture dishes. Refer to Table 5 for appropriate medium volumes.

Cells plated the day before should be 50% - 70% confluent on the day of transfection.

Table 4: Suggested Cell Culture Conditions for Transfection of Neuronal

Tissue Culture Dish	Number of Cells / Well
96-well	25-30 x 10^3
24-well	125-150 x 10^3
12-well	250-300 x 10^3
6-well	500-600 x 10^3
60 mm	1-1.5 x 10^6
100 mm	2.5-3 x 10^6

Table 5: Medium Volumes and DNA Amount for Various Culture Dishes

Tissue Culture Dish	DNA (µg)	Medium Volume (ml)
96-well	0.1-0.5	0.2
24-well	0.5-3	0.5
12-well	1-4	1
6-well	2-6	1.5
60 mm	6-8	2.5 8-12 5
100 mm		

2.6 Add fresh growth media as needed 24 hours post transfection. Depending on the cell type and promoter activity, the assay for the reporter gene can be performed 24 to72 hours following transfection.

For some cell types, the old media can be replaced with fresh media at this step.

The same protocol can be used to produce stably transfected cells: 48 to 72 hours post transfection, put the cells in fresh medium containing the appropriate selection antibiotic. It is important to wait at least 48 hours before exposing the transfected cells to the selection media. For some cell types it may be necessary to wait as long as 4 to 5 days before applying the selection condition.

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3. Transfection of Differentiated Post-Mitotic Neurons and Glial Cell Lines

3.1. Hydrate n-Fect lipid film at room temperature with 1.5 ml of the hydration buffer. Vortex for 3060 seconds at top speed. Store the hydrated reagent at 4ºC and vortex briefly before use.

3.2. Dilute the hydrated n-Fect reagent with serum-free medium. Refer to Table 6 for the appropriate volume of serum-free medium.

Table 6: Volumes of Transfection Reagents

DNA (µg)	Serum Free Medium for DNA (µl)	n-Fect (µl)	Serum Free Medium for n-Fect (µl)
0.5	15	5	10
1	25	10	15
2	50	20	30
4	75	40	35
6	100	60	40
8	150	80	70

3.3. Dilute the DNA with the serum free medium. Refer to Table 6 for the appropriate volume of serum-free medium.

Although n-Fect consistently delivers high transfection efficiencies, in order to obtain maximum efficiency in particular cell types, some optimization may be needed. The two critical variables are the ratio of n-Fect reagent to DNA and the quantity of DNA used. For optimization, first maintain a fixed ratio of n-Fect reagent to DNA, and then vary the DNA quantity over the suggested range. If necessary, optimize the ratio of n-Fect reagent to DNA by using 5 to 20 µl of reagent for each 1 µg of DNA. Use a low DNA quantity to optimize this ratio. Following this process, cell numbers can also be optimized. See the Appendix for examples.

3.4. Add the DNA solution to the diluted n-Fect Transfection Reagent. Incubate at room temperature for 5 to 10 minutes to allow the n-Fect /DNA complexes to form.

Do not incubate the DNA solution with the n-Fect Transfection Reagent for longer than 30 minutes

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3.5. Add your complexes directly to the cells growing in serum-containing culture medium. Refer to Table 7 for suggested cell number according to culture dishes size and cell types. Refer to Table 8 for appropriate medium volumes.

Cells plated the day before should be 50% - 70% confluent on the day of transfection.

Table 7: Suggested Cell Culture Conditions for Transfection of Differentiated Neurons and Glial Cells

Tissue Culture Dish	Cells / Well Diff. Neurons	Cells / Well Glial Cells
96-well	35 x 10^3	50 x 10^3
24-well	150 x 10^3	200 x 10^3
12-well	300 x 10^3	400 x 10^3
6-well	600 x 10^3	800 x 10^3
60 mm	1.5 x 10^6	2 x 10^6
100 mm	3 x 10^6	4 x 10^6

Table 8: Medium Volumes and DNA Amount for Various Culture Dishes

Tissue Culture Dish	DNA (µg)	Medium Volume (ml)
96-well	0.1-0.5	0.2
24-well	0.5-3	0.5
12-well	1-4	1
6-well	2-6	1.5
60 mm	6-8	2.5 8-12 5
100 mm		

3.6. 24 hours post transfection, add fresh growth media as needed. Depending on the cell type and promoter activity, the assay for the reporter gene can be performed 24 to72 hours following transfection.

For some cell types, the old media can be replaced with fresh media at this step. Also, the same protocol can be used to produce stably transfected cells: 48 to 72 hours post transfection, put the cells in fresh medium containing the appropriate selection antibiotic. It is important to wait at least 48 hours before exposing the transfected cells to the selection media. For some cell types it may be necessary to wait as long as 4 to 5 days before applying the selection condition.

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APPENDIX

Quality Control

To assure the performance of each lot of the n-Fect reagent, we pre-qualify the chemical synthesis of n-Fect lipid by mass spectrometry and thin layer chromatography. The final product is further tested by in vitro βgalactosidase transfection assay in NT2 neuronal precursor cell. Each lot shall have an acceptance specification of >70% of the activity of the Reference lot.

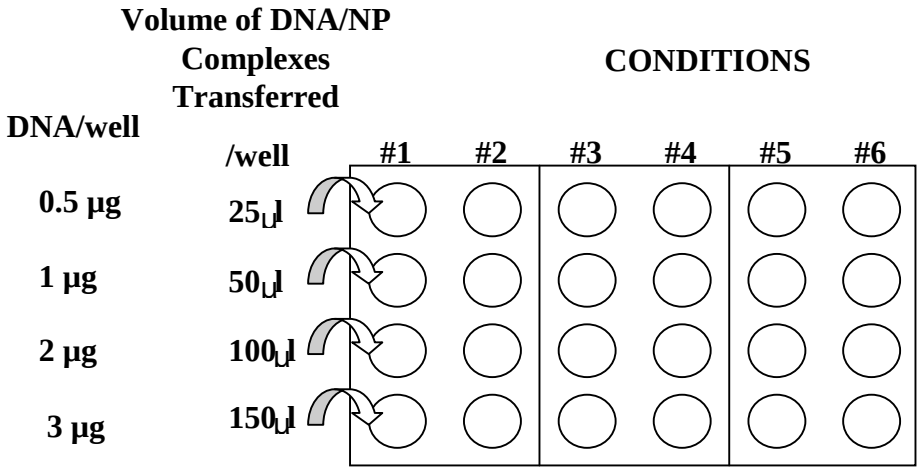
Examples of Optimization of Transfection Conditions

1. Optimization conditions for primary neuron transfection in 24-well plates

Follow the general protocol to prepare the DNA/n-Fect complexes. We **do not recommend** using the DNA Diluent for primary neurons.

Condition	DNA dilutions in serum free medium	n-Fect dilutions in serum free medium	Total Volume	Final DNA Concentration
1	10 µg in 250 µl	25 µl in 225 µl (Vt = 250 µl)	500 µl	20 µg/ml
2	"	50 µl in 200 µl (Vt = 250 µl)	"	"
3	"	75 µl in 175 µl (Vt = 250 µl)	"	"
4	"	100 µl in 150 µl (Vt = 250 µl)	"	"
5	"	125 µl in 125 µl (Vt = 250 µl)	"	"
6	"	150 µl in 100 µl (Vt = 250 µl)	"	"

Add the appropriate volume of complexes solution directly to your cells as illustrated below.



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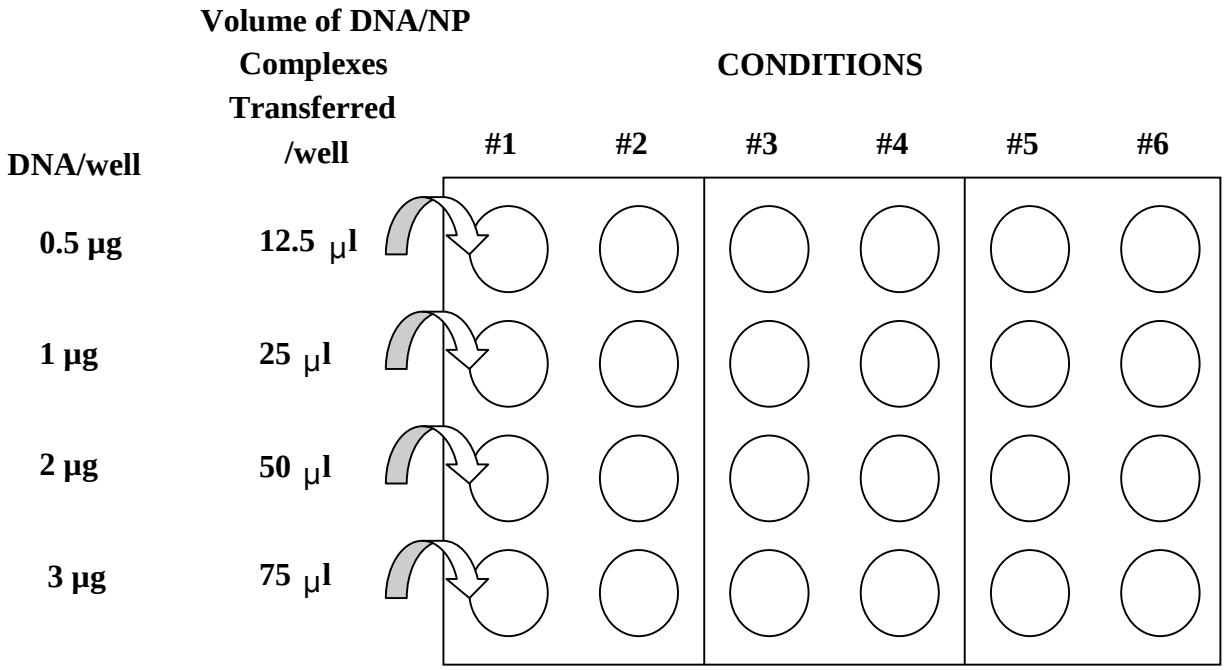
2. Optimization conditions for neuronal cell line transfection in 24-well plates

Follow the general protocol to prepare the DNA/n-Fect complexes. We **recommend** using the DNA Diluent for neuronal cell lines such as NT2.

Condition	DNA Diluent	n-Fect dilutions in serum free medium	Total Volume	Final DNA Concentration
1	10 µg in 125 µl	12.5 µl in 112.5 µl (Vt = 125 µl)	250 µl	40 µg/ml
2	"	25 µl in 100 µl (Vt = 125 µl)	"	"
3	"	50 µl in 75 µl (Vt = 125 µl)	"	"
4	"	75 µl in 50 µl (Vt = 125 µl)	"	"
5	"	100 µl in 25 µl (Vt = 125 µl)	"	"
6	"	125 µl n-Fect	"	"

Add the appropriate volume of complexes solution directly to your cells as illustrated below.



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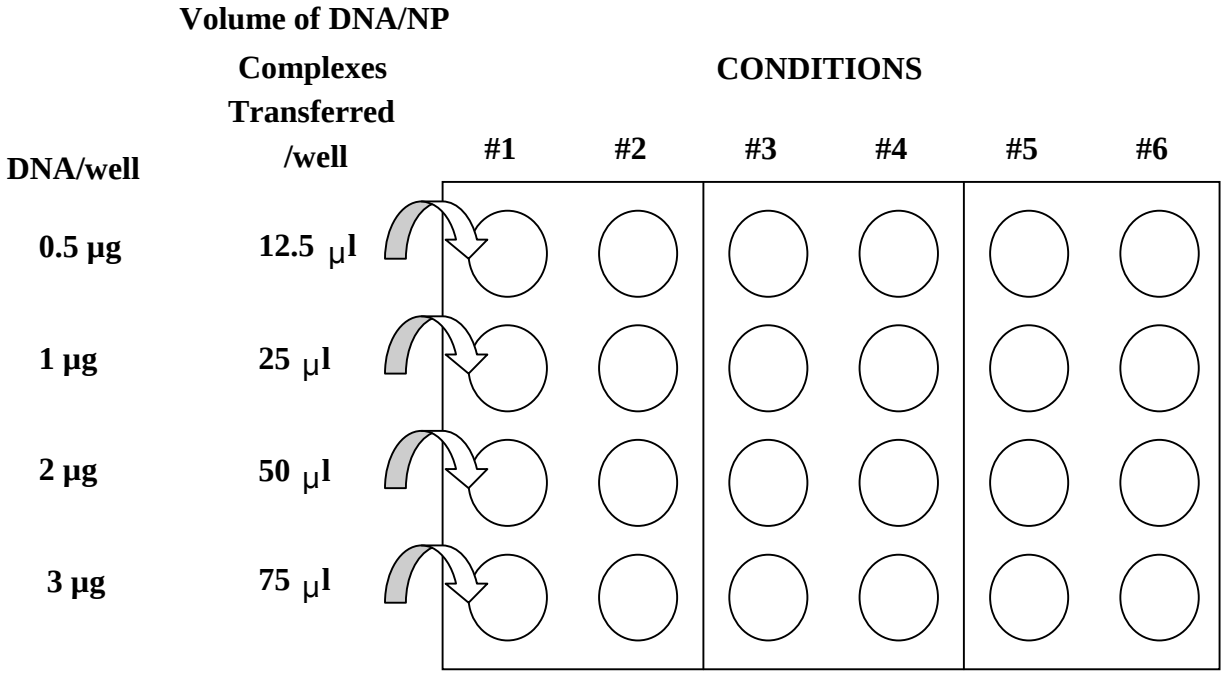
3. Optimization conditions for differentiated post-mitotic neurons and glial cell line transfection in 24well plates

Follow the general protocol to prepare the DNA/n-Fect complexes. We **do not recommend** using the DNA Diluent for differentiated post-mitotic neurons and glial cells.

Condition	DNA dilutions in serum free medium	n-Fect dilutions in serum free medium	Total Volume	Final DNA Concentration
1	10 µg in 250 µl	50 µl in 200 µl (Vt = 250 µl)	500 µl	20 µg/ml
2	"	75 µl in 175 µl (Vt = 250 µl)	"	"
3	"	100 µl in 150 µl (Vt = 250 µl)	"	"
4	"	125 µl in 125 µl (Vt = 250 µl)	"	"
5	"	150 µl in 100 µl (Vt = 250 µl)	"	"
6	"	200 µl in 50 µl (Vt = 250 µl)	"	"

Add the appropriate volume of complexes solution directly to your cells as illustrated below.



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