

NEOFECTAMINE 3000 [In Vitro Transfection Reagent]

The protocol for transfection of mammalian cells

Store at 4°C

This product is for research use only.

Broad Transfection Spectrum for Mammalian Cell Types

Introduction to NEOFECTAMINE 3000 Transfection Reagent

Product Overview

NEOFECTAMINE 3000 Transfection Reagent is a strong transfection reagent that ensures effective and reproducible transfection with invisible toxicity. It is formulated by unique chemistry (covalently cross-linking cationic lipids with polymer), giving rise to exceptional transfection efficiency with distinguishable features in comparison of other types of reagents. NEOFECTAMINE 3000 Transfection Reagent was shown to deliver genes to various established cell lines as well as primary cells including HEK293, 293T, 293E, CHO, COS1, HeLa, NIH 3T3, insect cell lines (Sf9 and Sf21) and a variety of other eukaryotic cell lines. NEOFECTAMINE 3000 Transfection Reagent, 1.0 ml, is sufficient for 300 to 600 transfections in 24 well plates or 50 to 100 transfections in 6 well plates.

Cell Lines	Transfection Efficiency (% GFP)	Cell Lines	Transfection Efficiency (% GFP)
3LL	51%	K562	22%
B16-F10	72%	L929	52%
BAEC	52%	MCF-7	51%
BHK-21	86%	MDCK	52%
Ca Ski	78%	Neuro 2A	72%
CaCo2	32%	NIH 3T3	88%
CHO	83%	PC12	34%
HCS-2/8	42%	SH-SY5Y	14%
HEK-293	80-90%	SiHa	62%
HeLa	81%	SKOV3	52%
HLMEC	50-65%	HUVEC	51%
H-MVEC	49%	IGROV1	21%
Huh-7D12	22%	Jurkat	5%-15%
ATT20	38%	6CSFMEo	73%
SK-N-SH	20%	WEHI 231	25%
McArdle 7777	60%	SAOS-2	52%
Hep3D	78%	SN56	69%
SHEP	71%	MC3T3-E1	80%
MDA-MB-231	38%	BT474	46%
3T3 -442A	10%	C 2C 12	46%
COS-7	60-70%	Primary mouse keratinocytes	40-50%
CV-1	53%	Primary human pre-adipocytes	40-50%
D 407	62%	Primary human skin fibroblasts	25%
DHD Pro.b	51%	Primary mouse embryonic fibroblast	20%
LS180	46%	Primary melanocyte	46%
A549	78%	Hela-S3	78%

Application

- Deliver DNA/RNA into mammalian, insect cells
- Enhance (up to 200 times higher efficiency) virus infection of hard-to-transfect cells, e.g., replication-deficient Adenovirus

Features

- Low cytotoxicity for most of tumor cell lines and primary cells
- High transfection efficiency for a broad range of cell types
- Efficient transfection with or without serum
- Simple and robust transfection procedure
- Effectively transfects both adherent and suspension cell cultures
- Exceptional high titers of virus production
- Excellent for long DNAs (up to 25 kb), for single or multiple DNA transfections, and for hard-to-transfect cells

Storage Condition

Store at 4 °C. If stored properly, the product is stable for 12 months or longer.

India Contact:

Life Technologies (India) Pvt. Ltd.

306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034 (INDIA). Ph: +91-11-42208000, 42208111, 42208222, Mobile: +91-9810521400, Fax: +91-11-42208444
Email: customerservice@lifetechindia.com Website: www.lifetechindia.com

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Procedures for Transfecting Mammalian Cells:**1. For Adherent Cells****Cell Seeding (see Table 1):**

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal 80~90% confluency at the time of transfection. Serum-free DMEM medium or serum-containing medium (less than 5% serum) without antibiotics is changed to replace complete serum-containing culture medium 1 hour before transfection.

Note: High serum levels (>5%) have a moderate inhibitory effect on NEOFECTAMINE 3000 -mediated transfections. Maximal transfection efficiencies are observed in the absence of serum. Depending upon the cell type, the presence of serum <5% may sometimes improve the overall levels of recombinant protein expression.

Table 1. A guideline for seeding adherent cells prior to transfection in different culture formats.

Culture Dish	Surface Area(cm ²)	Number of Cells to Seed
T175 flask	175	0.7-1.4 × 10 ⁷
T75 flask	75	3.0 - 6.0 × 10 ⁶
100 mm dish	58	2.2 – 4.4 × 10 ⁶
60 mm dish	21	0.9 - 1.8 × 10 ⁶
35mm dish	9.6	3.5 – 7.0 × 10 ⁵
6-well plate	9.6	4.0 – 8.0 × 10 ⁵
12-well plate	3.5	1.5 – 3.0 × 10 ⁵
24-well plate	1.9	0.8 – 1.6 × 10 ⁵
48-well plate	1	4.0 – 8.0 × 10 ⁴
96-well plate	0.3	1.2 - 2.4 × 10 ⁴

Preparation of NEOFECTAMINE 3000 DNA Complex and Transfection Procedures

For different cell types, the optimal ratio of NEOFECTAMINE 3000 (μL) : DNA (μL) varies from 1:1 to 3:1. We recommend the NEOFECTAMINE 3000 (μL) : DNA (μL) ratio of 3:1 as a starting point which usually gives satisfactory transfection efficiency with invisible cytotoxicity. To ensure the optimal size of complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and NEOFECTAMINE 3000 reagent.

The following protocol is given for transfection in 24-well plates, refer to **Table 2** for transfection in other culture formats. The optimal transfection conditions for a majority of adherent cell lines, as well as a general starting point for optimization are given in the standard protocol described below.

- For each well, dilute 1 μg of DNA into 50 μl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.
- For each well, dilute 3 μl of NEOFECTAMINE 3000 solution into 50 μl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.
- Add the 50 μl NEOFECTAMINE 3000 solution to the 50 μl DNA solution all at once. (**Important: do not mix the solutions in the reverse order !**)
- Vortex- mix the solution immediately and spin down briefly to bring drops to the bottom of the tube.
- Incubate for 10 minutes at room temperature.
- Add the 100 μl NEOFECTAMINE 3000 / DNA mixture dropwise onto the medium in each well and homogenize the mixture by gently swirling the plate.
- For maximal transfection efficiency, change the medium to complete serum containing medium 4-5 hours post addition of NEOFECTAMINE 3000 /DNA complex.
- Check transfection efficiency 24 to 48 hours post transfection.

2. For Suspension Cells

The following protocol is given for transfection in 6-well plate. The protocol can be scaled up or down according to culture volume.

Cell Seeding: Suspension cells are typically seeded the day of the transfection at a density of 0.5–1.0 × 10⁶ cells per ml of culture. For optimal transfection conditions with NEOFECTAMINE 3000, seed the number of cells adapted to the culture vessel format according to **Table 3**.

Table 2. Recommended Amounts for Different Culture Vessel

Culture Dish	Culture Volume (mL)	Plasmid DNA (ug)	Diluent (mL)	Neofectamine 3000
6-well plate	3	2-4	0.3	6-12
35 mm dish	3	2-4	0.3	6-12
60 mm dish	5	6-12	0.5	18-36
100 mm dish	10	12-24	1	36-72
T75 flask	15	18-36	1.5	54-108
250-mL flask	50	50-100	2.5	150-300

Table 3. Recommended number of suspension cells to seed. Culture Dish Number of Cells

Culture Dish	Number of Cells
96-well plate	2 × 10 ⁴ - 5 × 10 ⁴
48-well plate	5 × 10 ⁴ - 1 × 10 ⁵
24-well plate	1 × 10 ⁵ - 2 × 10 ⁵
6-well plate	2 × 10 ⁵ - 5 × 10 ⁵
35 mm dish	5 × 10 ⁵ - 2 × 10 ⁶
60 mm dish	2 × 10 ⁶ - 5 × 10 ⁶
100 mm dish	5 × 10 ⁶ - 1 × 10 ⁷

NEOFECTAMINE 3000 DNA Complex Preparation and Transfection**Procedures:**

The optimal ratio of NEOFECTAMINE 3000 / DNA is of 3/1 (3 μl of NEOFECTAMINE 3000 Reagent is used per 1 μg of plasmid DNA). We recommend using serum-free DMEM with High Glucose to dilute DNA and NEOFECTAMINE 3000 Reagent to ensure the optimal size of complex particles.

The following protocol is given for transfection in 6-well plates.

- For each well, dilute 2 – 3 μg of DNA into 100 μl of serum-free DMEM with high glucose. Vortex gently and spin down briefly.
- For each well, dilute 6 – 9 μl of NEOFECTAMINE 3000 solution into 100 μl of serum-free DMEM with high glucose. Vortex gently and spin down briefly.
- Add the 100 μl NEOFECTAMINE 3000 solution to the 100 μl DNA solution all at once (**important: do not mix the solutions in the reverse order**)
- Vortex- mix the solution immediately and spin down briefly to bring drops to the bottom of the tube.
- Incubate for 10 minutes at room temperature.
- Add the 200 μl NEOFECTAMINE 3000 / DNA mixture drop- wise onto the serum containing medium in each well, homogenize the mixture by gently swirling the plate.
- Incubate at 37 °C and 5% CO₂ in a humidified atmosphere.
- Transfection experiments are usually stopped after 24 to 48 hours and gene activity assessed. Cells growing in suspension are collected by centrifugation at 800 xg and then resuspended in the desired medium or buffer.

Storage: NEOFECTAMINE 3000 DNA In Vitro Transfection Reagent is stable for up to 18 months at 4°C. This item is shipped at ambient temperature.

India Contact:**Life Technologies (India) Pvt. Ltd.**

306, Aggarwal City Mall, Opposite M2K Pitampura,

Delhi – 110034 (INDIA).

Ph: +91-11-42208000, 42208111, 42208222

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Fax: +91-11-42208444

Email: customerservice@lifetechindia.comWeb: www.lifetechindia.com