ExCell Bio
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ExCell Bio

NanoFectin Transfection Reagent

User Manual

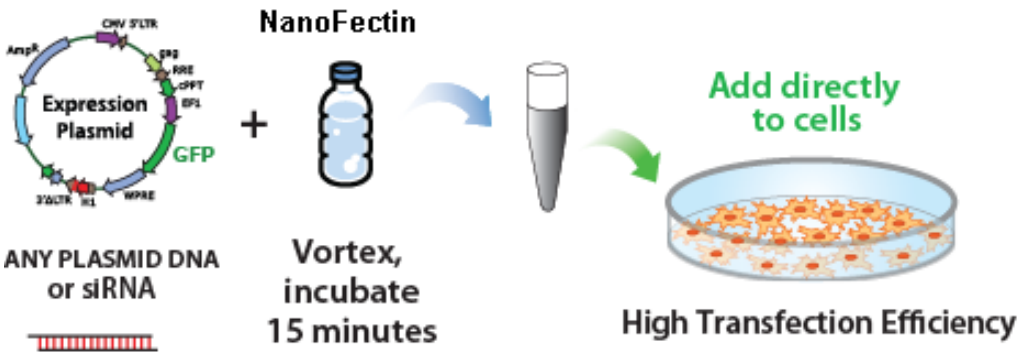
Catalog Number EMB750A-1 1mL

Introduction

Nanofectin Nanotechnology-based Transfection Reagent delivers more DNA and siRNAs to cells than the leading lipid-based transfection kits.
Nanofectin is a powerful, broadly applicable transfection reagent for effective and reproducible transfections.

The Nanofectin reagent self-assembles nanoparticles in the presence of DNA and RNA. These complexes are readily taken up by target cells for efficient gene delivery. No media changes are required as Nanofectin works in the presence of antibiotics and serum. Easy-to-use protocol with rapid, one-step incubation for 15 minutes before adding directly to target cells makes Nanofectin well-suited for high-throughput transfection experiments

Flowchart

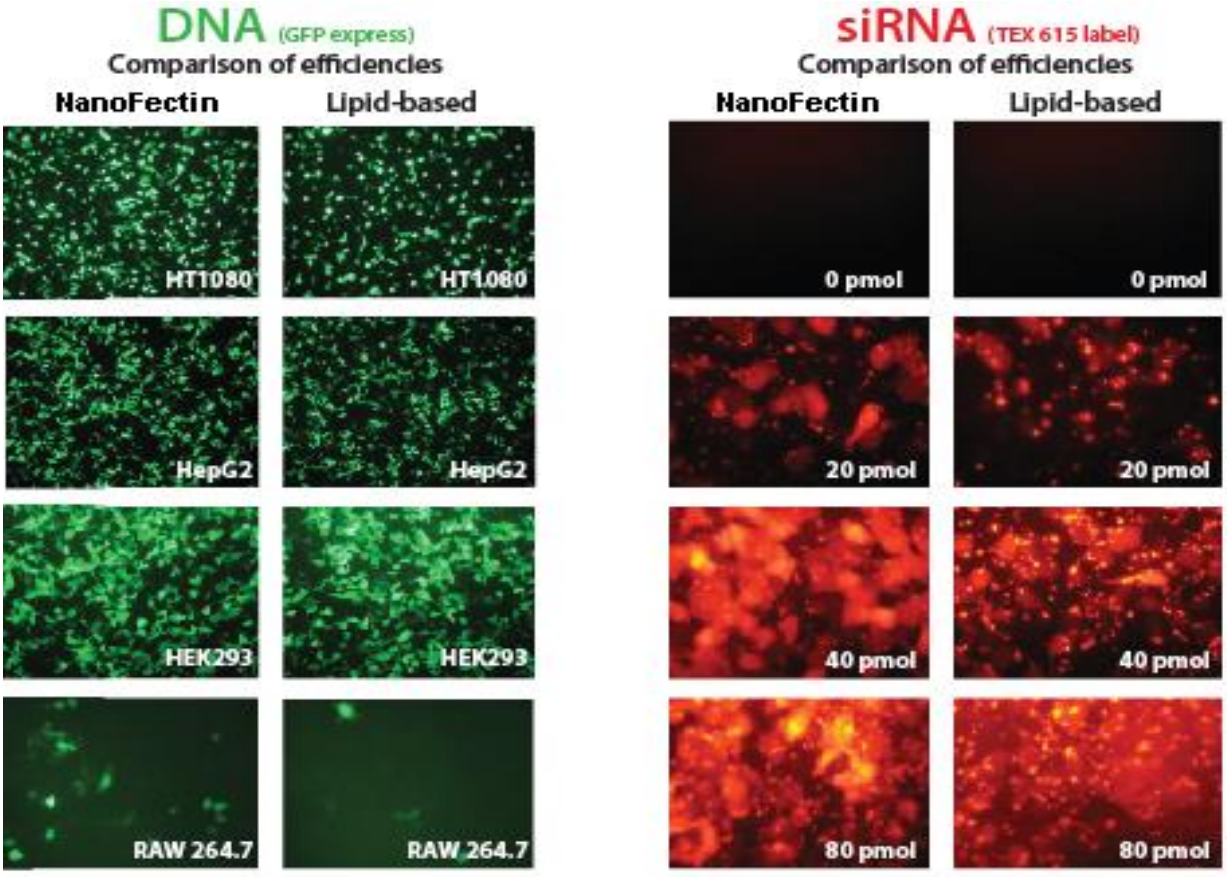


Advantages

- Highly effective transfection technology - works with most cell types
- Cost-effective alternative to lipid-based products
- Nano-based gene delivery with low toxicity
- Rapid 15 minute protocol makes Nanofectin ideal for high-throughput transfections
- Works with both Plasmid DNA and siRNAs
- Package virus with high titer, low cost and simple procedure

Data Analysis

Comparison of Transfection Efficiency.



Application

The Nanofectin reagent self-assembles nanoparticles in the presence of DNA and RNA in the presence of antibiotics and serum.

Shipping & Storage

Shipping： Room temperature

Storage ： 4°C

Shelf Life： 12 months from date of receipt with proper storage

Protocol

Important Guidelines for Transfection

For high Transfection efficiency and lower toxicity, transfect cells at high density. 50-80% confluency is highly recommended

Step I. Cell Seeding:

Cells should be plated 18 to 24 hours prior to transfection so that the cell density reaches to 50~80% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 2 hours before transfection.

Step II. Preparation of Nanofectin/DNA Complex and Transfection Procedures:

The following protocol is given for transfection in a 24-well plate, refer to Table 1 for transfection in other culture formats.

1. For each well, add 0.5 ml of complete medium with serum and antibiotics (antibiotic does not influence the result) freshly 2 hours before transfection.
2. For each well, dilute 0.5 μg of DNA into an Eppendorf tube with 50 μl of serum-free DMEM, and Mix gently.
3. Add 1 μl of Nanofectin reagent into the same tube. Vortex 5-10 seconds and spin down briefly to bring drops to the bottom of the tube.
4. Incubate for ~15 minutes at room temperature to allow Nanofectin/DNA complexes to form.
5. Add the 50 μl Nanofectin/DNA mix drop-wise to the cells in each well and homogenize by gently swirling the plate.
6. Return the plates to the cell culture incubator.
7. Check transfection efficiency 24 to 48 hours post transfection.

Table 1. Recommended Amounts for Different Culture

Culture Dish	Surface Area (cm^2)	Cell Number	Volume (ml)	Plasmid (μg)	Nano-Fectin(μl)	Diluent Volume (μl)
96-Well	0.3	1-1.7x10^4	0.1	0.1	0.2	10
48-Well	1	2.5-5x10^4	0.25	0.25	0.5	20
24-Well	2	0.5-1x10^5	0.5	0.5	1-2	50
12-Well	4	1-2x10^5	1	1	2-4	100
6-Well/35 mm	9.5	2-4x10^5	2	2.5	4-8	200
60 mm/T25	28	5-10x10^5	5	4-8	12-24	300
100 mm/T75	79	1.5-3x10^6	10	10-20	30-40	500
150 mm/T150	153	5-9x10^6	20	25-40	40-60	1000

Note:

For different cell types, the optimal ratio of Nanofectin (μL): DNA (μg) is around 2:1. We recommend the Nanofectin (μL):DNA (μg) ratio of 2:1 as a starting point which usually gives satisfactory transfection efficiency with invisible cytotoxicity, however the amount of Nanofectin may be adjusted

from 1 to 4 µl per µg of DNA depending on the cell line to be transfected. To ensure the optimal size of Nanofectin/DNA complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and Nanofectin Reagent.

Procedures for Packaging Lentivirus

1. Seed 7-8X10^6 of 293 TN or 293 FT cell in one 150 cm^2 Cell culture plate [#1] in 20ml of normal culture medium (without antibiotics) 18 to 24 hours prior to transfection, so that the cell density reaches to 60~80% confluency at the time of transfection.
2. Add 1-1.6ml of DMEM[#2] (serum free) to an autoclaved 2 ml Eppendorf tube,
3. Add 45µl of pPACKH1 (SBI,Cat#LV500A-1) and 4.5µg of plasmid [#3] (construct based on lentivector) into DMEM. Mix by pipetting.
4. Then add 55µl of Nanofectin [#4] (Cat# EMB750A) into DMEM-Plasmids mixture. Mix well by vortex 10 seconds.
5. Incubate DMEM-Plasmids-Nanofectin mixture at room temperature for 15 minutes.
6. Add DMEM-Plasmids-Nanofectin mixture drop-wise into the dish, and swirl the dish to allow the mixture disperse evenly in the plate.
7. Return the dish to cell culture incubator at 37 °C with 5 % of CO_2.
8. Change fresh medium at 12-24 hours after transfection (optional).
9. Collect medium that contains lentivirus at 48 hours and 72 hours after transfection into a 50-ml sterile, capped conical centrifuge tube. Centrifuge at 3000rpm for 15 minutes at room temperature to pellet cell debris. Transfer the viral supernatant into a new tube.
10. For fresh viral supernatant, aliquot the supernatant into sterile 1.5-ml microfuge tubes and store them at -80 °C.
11. To concentrate virus, add a quarter volume of Lenti-Virus Precipitation Solution (Cat# EMB810A) to the viral supernatant (volume of Lenti-Virus vs. volume of viral supernatant = 1:4) and mix thoroughly. Put the mixture to 4 °C refrigerator over night and spin the virus pellet down next day. Please refer the user manual for details.

- **#1. If use 10cm plate, seed 3-4X10^6cells/dish in 9 ml of normal culture medium without antibiotics.**
- **#2. 0.8ml of serum free DMEM can be used for 10 cm plate.**
- **#3. 20ul of pPACKH1 and 2 ug of plasmid will be used to 10 cm plate.**
- **#4. 24 ul of NanoFectin (ExCell Bio, Cat # EMB750A) can be used to 10cm plate.**

Protocols for SiRNA Transfection on 24-well Plate Format

1. Seed 0.5-1X10^5 cells/well in 0.5 ml of culture medium 16-24 hours before transfection. The cell density reaches 40-60% confluency according to the cells' growth rate and the purpose of the experiment.
2. Prepare SiRNA: Dilute SiRNA with RNase-Free Buffer (pH7.5) to the work concentration of 10 µM. Heat solution to 94 °C for 2 minutes. Cool it at room temperature. Product will be fully resuspended in a stable, double-stranded form. Aliquot enough amount for transfection. Store the remaining at -20 °C.
3. Prepare Nanofectin-SiRNA complex: Add serum-free DMEM 100 µl to an Eppendorf tube. Add SiRNA 20, 40, and 80 pmol to each tube to test the optimist amount to your cells. Add 1 µl of Nanofectin to each tube. Mix well with vertex. Incubate at room temperature for 15 minutes.

4. Add the Nanofectin-SiRNA mixture into each well of cell drop-wisely.
5. Return the plate to cell culture incubator at 37 °C with 5 % of CO_2. Incubate for 1-3 days depending on the purpose of experiment.