NanoFect™ Transfection Reagent Protocol

Catalogue number: NF100; Size: 1ml

Product Descriptions

NanoFect™ Transfection Reagent is a unique blend of polymers that delivers more DNA and siRNAs to cells than the leading lipid-based transfection kits. NanoFect™ is a powerful, broadly applicable transfection reagent for effective and reproducible transfections. The Nano-Fect 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 NanoFect™ works in the presence of antibiotics and serum. The easy-to-use protocol with rapid, one-step incubation for 15 minutes before adding directly to target cells makes NanoFect™ well-suited for high-throughput transfection experiments.

Important Guidelines for Transfection

For high transfection efficiency and lower toxicity, transfect cells at high density. 70-80% confluence is highly recommended.

Protocol

Cells should be plated 18 to 24 hours prior to transfection so that the cell density reaches 70~80% confluence at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 2 hours before transfection.

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 normal growth medium (antibiotic does not influence the result) freshly 2 hours before transfection.
2. For each well, dilute 0.5 µg of DNA in 50 µl of DMEM without serum, and mix gently.
3. Add 1.5 µl of NanoFect™ reagent (ALSTEM, Cat. # NF100) into another tube with 50 µl of DMEM without serum and mix gently.
4. Add NanoFect™/DMEM into DNA/DMEM solution. Mix by vortexing for 5-10 seconds.
5. Incubate for ~15 minutes at room temperature to allow for NanoFect™/DNA complexes self-assembly.
6. Add the 100 µl NanoFect™/DNA mix drop-wise to the cells in each well and homogenize by gently swirling the plate.
7. Return the plates to the cell culture incubator.
8. Check transfection efficiency 24 to 48 hours post transfection.

ALSTEM
CELL ADVANCEMENTS
2600 Hilltop Drive, B/C 328, Richmond, CA 94806
www.alstembio.com Tel: (510) 708-0096 Email: info@alstembio.com

Table 1. Recommended Amounts for Different Culture

Culture Dish Surface	Area (cm^2)	Cell Number	Medium Volume (ml)	Plasmid(µg)	NanoFect (µl)	Diluent Volume (µl)
96-well	0.3	1-1.5x10^4	0.1	0.1	0.3	10
48-well	1	2.5-5x10^4	0.25	0.25	0.75	20
24-well	2	0.5-1x10^5	0.5	0.5	1.5	50
12-well	4	1-2x10^5	1	1	3	100
6-Well/35 mm	9.5	2-4x10^5	2	2.5	7.5	200
60 mm/T25	28	5-10x10^5	5	6-8	15-24	300
100 mm/T75	79	1.5-3x10^6	10	15-20	40-60	500
150 mm/T150	153	5-9x10^6	20	25-40	65-120	1000

Note: For different cell types, the optimal ratio of NanoFect (µL): DNA (µg) is around 3:1. We recommend the NanoFect (µL):DNA (µg) ratio of 2:1 as a starting point which usually gives satisfactory transfection efficiency with invisible cytotoxicity, however the amount of NanoFect may be adjusted from 2 to 4 µl per µg of DNA depending on the cell line to be transfected. To ensure the optimal size of NanoFect/DNA complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and NanoFect Reagent.

Packaging Lentivirus

1. 18 to 24 hours prior to transfection, seed 6-8 x 10_6 HEK293FT cells per 150 mm_2 cell culture plate in 20 ml of culture medium containing 10% FBS (without antibiotics) so that the cell density reaches to 70~90% confluence at the time of transfection.
2. Add 1-1.6 ml of DMEM (serum free) to a 2 ml tube.
3. Add 45 µl of lentiviral packaging mix and 4.5 µg of your plasmid construct to the same tube of DMEM. Mix by pipetting.
4. Then add 55 µl of NanoFect™ into DMEM-Plasmid mixture. Mix well by vortex 10 seconds.
5. Incubate DMEM-Plasmid-NanoFect™ mixture at room temperature for 15 minutes.
6. Add DMEM-Plasmid-NanoFect™ mixture drop-wise into the dish, and swirl the dish to disperse evenly in the plate.
7. Return the dish to cell culture incubator at 37° C with 5% CO_2.
8. Change to fresh medium 12-24 hours after transfection.

9. Collect medium that contains lentiviruses at 48 hours and 72 hours after transfection into a 50-ml sterile, capped conical centrifuge tube. Centrifuge at 3000 rpm for 15 minutes at room temperature to pellet cell debris. Filter the viral supernatant through 0.45-µm filter.
10. For fresh filtered viral supernatant, aliquot the supernatant into sterile 1.5-ml tubes and store them at -80° C.
11. To concentrate virus, add a quarter volume of 5X Lentivirus Precipitation Solution (ALSTEM, Cat. # VC100) to the viral supernatant (volume of Lentivirus Precipitation Solution vs. volume of viral supernatant = 1:4) and mix thoroughly. Put the mixture in a 4° C refrigerator overnight and spin the virus pellet down the next day. Please refer to the user manual for details.

Note: If you use 100 mm plates, seed 4 x 10_6 cells/ dish in 10 ml culture medium without antibiotics.
In step 2, add 0.8 ml of serum free medium per 100 mm plate.
In step 3, add 20 µl of Lentiviral packaging mix and 2 µg plasmid per 100 mm plate.
In step 4, add 24 µl of NanoFect™ per 100 mm plate.

Packaging Retrovirus

1. 18 to 24 hours prior to transfection, seed 8 X 10_6 293FT cells per 150 mm_2 cell culture plate in 20 ml of normal culture medium (without antibiotics) so that the cell density reaches to 70~90% confluence at the time of transfection.
2. Add 1.2 ml of DMEM (serum free) to a 2 ml tube.
3. Add 20 µl of retroviral packaging mix and 10 µg of your plasmid construct to the same tube of DMEM. Mix by pipetting.
4. Then add 50 µl of NanoFect™ into DMEM-Plasmid mixture. Mix well by vortex 10 seconds.
5. Incubate DMEM-Plasmid-NanoFect™ mixture at room temperature for 15 minutes.
6. Add DMEM-Plasmid-NanoFect™ mixture drop-wise into the dish, and swirl the dish to disperse evenly in the plate.
7. Return the dish to cell culture incubator at 37° C with 5% CO_2.
8. Change to fresh medium 12-24 hours after transfection.
9. Collect medium that contains retroviruses at 48 hours and 72 hours after transfection into a 50-ml sterile, capped conical centrifuge tube. Centrifuge at 3000 rpm for 15 minutes at room temperature to pellet cell debris. Filter the viral supernatant through 0.45 µm filter.
10. Aliquot the filtered fresh viral supernatant into sterile 1.5-ml tubes and store them at -80° C.
11. To concentrate virus, add a quarter volume of 5X Retrovirus Concentration Solution (Cat. # VC200) to the viral supernatant (volume of Retrovirus Concentration Solution vs. volume of viral supernatant = 1:4) and mix thoroughly. Put the mixture in a 4° C refrigerator overnight and spin the virus pellet down next day. Please refer to the user manual for details.

Note: If you use 100 mm plates, seed 4 x 10_6 cells/ dish in 10 ml culture medium without antibiotics.
In step 2, add 0.8 ml of serum free medium per 100 mm plate.
In step 3, add 20 µl of Retroviral packaging mix and 5 µg plasmid per 100 mm plate.
In step 4, add 20 µl of NanoFect™ per 100 mm plate.