ProMab Biotechnologies, Inc.

Protocol

PACKAGING LENTIVIRUS

OVERVIEW

NanoFect™ Transfection Reagent (cat. # NF100, ProMab Biotechnologies) 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-o-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.

PACKAGING PROCEDURE

1. 18 to 24 hours prior to transfection, seed 6-8X10^6 HEK293FT cells in per 150 mm2 cell culture plate in 20ml of culture medium containing 10% FBS (without antibiotics) so that the cell density reaches to 70~90% confluent at the time of transfection.
2. Add 1 to 1.6 ml of DMEM (serum free) to a 2 ml tube.
3. Add 45 μl of lentiviral packaging mixture 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 (cat.# VC100, ProMab Biotechnologies) to the viral supernatant (volume of Lentivirus Precipitation Solution vs. volume of viral supernatant = 1:4) and mix thoroughly. Put the mixture to 4°C refrigerator overnight and spin the virus pellet down next day. Please refer the user manual for details.

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ADDITIONAL NOTES

If you use 100 mm plates, seed 4X10^6 cells/ dish in 10ml culture medium without antibiotics.
In step 2, add 0.8ml of serum free medium per 100 mm plate.
In step 3, add 20µl of Lentivirus packaging mix and 2 µg plasmid per 100 mm plate.
In step 4, add 24 µl of NanoFect per 100 mm plate.

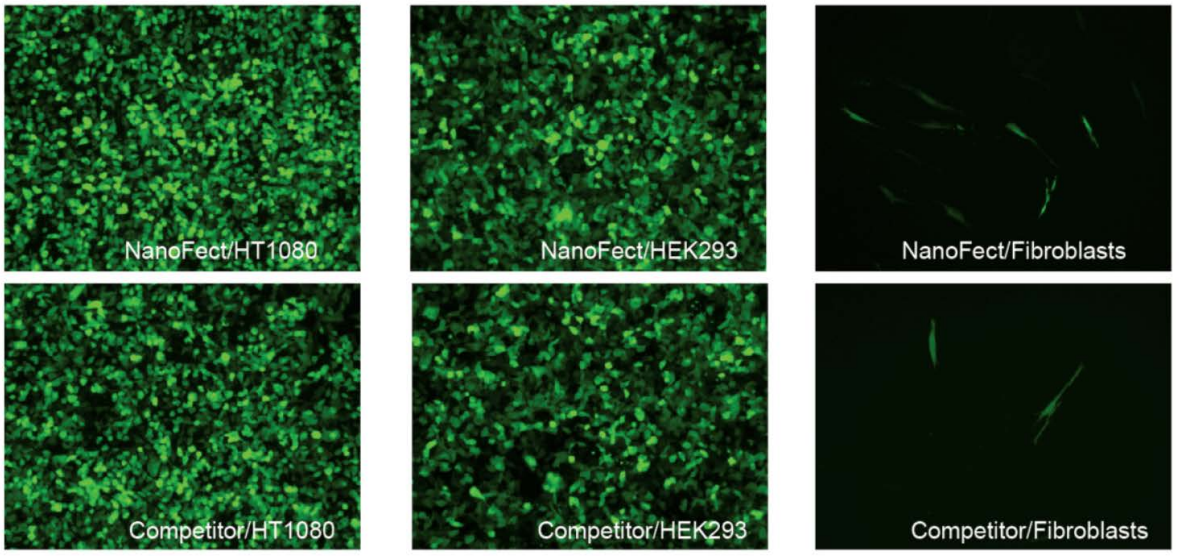

Figure 1. Comparison of transfection efficiency of NanoFect with leading competitor shows higher yield of GFP expressing cells and greater fluorescent intensity under microscopy.