Protocol

PACKAGING RETROVIRUS

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 $8X10^6$ 293FT cells in per 150 mm2 cell culture plate in 20 ml of normal culture medium (without antibiotics) so that the cell density reaches to 70~90% confluency 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 mixture 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 3000rpm 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, ProMab Biotechnologies) to the viral supernatant (volume of Retrovirus Concentration 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.

Additional Products and Services:

- Mouse Monoclonal Antibody
- Rat Monoclonal Antibody
- Rabbit Monoclonal Antibody
- Human Monoclonal Antibody
- Polyclonal Antibody
- Antibody Sequencing
- Hybridoma Sequencing
- CAR T-cells
- Lentivirus production
- Cancer Stem Cells
- Specialty Cell Culture Media
- T-cell Expansion beads

Ask about our full line of CRO services to provide supplemental assistance or the entire support necessary to complete your project on time and with the data you need to move forward.

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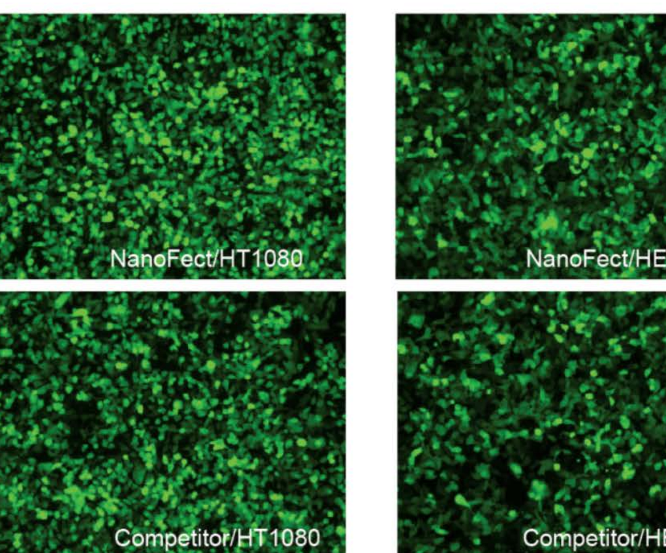
ADDITIONAL NOTES

If you use 100 mm plates, seed 4X10^6 cells/ dish in 10 ml normal culture medium without antibiotics.
In step 2, add 0.8ml of serum free medium per 100 mm plate.
In step 3, add 20µl of Retrovirus packaging mix and 5 µg plasmid per 100 mm plate.
In step 4, add 20 µl of NanoFect per 100 mm plate.



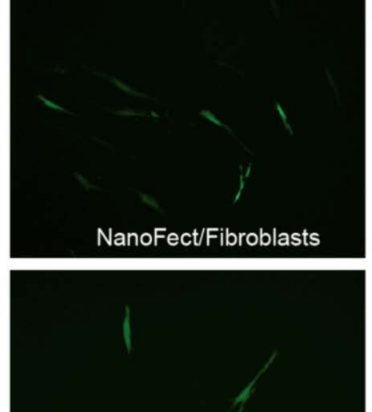


Figure 1. Comparison of transfection efficiency of NanoFect with leading competitor shows higher yield of GFP expressing cells and greater fluorescent intensity under microscopy.