Cat # SL100488 Store at 4 °C

GenJet™ Plus In Vitro DNA Transfection Reagent

----- A Protocol for Transfections of Bacmids Into Sf9 Cells

- ☐ 100 µl
- ☐ 500 µl
- ☐ 1000 µl

SignaGen® Laboratories
15875 Gaither Drive
Gaithersburg, MD 20877
FAX. 301-560-4919
TEL. 301-330-5966
Tool Free. 1-(866)-918-6812
Email: info@signagenlabs.com
Web: www.signagenlabs.com

This product is for laboratory research ONLY and not for diagnostic use

Introduction:

GenJet™ Plus DNA In Vitro Tranfection Reagent is a powerful transfection reagent that ensures effective and reproducible transfection with low toxicity. GenJet™ is formulated by covalently cross-linking cationic liposome with polymer, giving rise to exceptional transfection efficiency and extremely low toxicity. GenJet™ Plus was shown to deliver genes to various established cell lines as well as primary cells. GenJet™ reagent efficiently transfects HEK293, 293T, 293E, CHO, COS1, HeLa, NIH 3T3, insect cell lines (Sf9 and Sf21) and a variety of other eucaryotic cell lines. GenJet™ Plus reagent, 1.0 ml, is sufficient for 300 to 600 transfections in 24 well plates or 50 to 100 transfections in 6 well plates.

Features:

- Exceptional transfection efficiency of a broad range of cell types
- Very low cytotoxicity
- Efficient transfection with or without serum
- Simple protocols for suspension or adherent cells
- High levels of recombinant protein production
- Inexpensive transfection reagent
- Simple, robust transfection procedure
- Effectively transfects both adherent and suspension cell cultures

Procedures for Transfecting Bacmids into Sf9 Cells:

1. Count Sf9 cells, and adjust cell density to 5 x10^5 cells/ml in unsupplemented SF900II media
2. Seed 2 ml of cell suspension per well (1 x 10^6 cells/well).
3. Label 2 wells as "negative control", 2 wells as "1 µg DNA", and 2 wells as "2 µg DNA"
4. Incubate dishes at 27° C for 30-60 minutes (enough time to allow the cells to attach to the bottom of the wells).
5. Aliquot 500 µl of sterile diluent (150 mM NaCl) into three 1.5 ml Eppendorf tubes. Label the tubes "0", "1 µg", and "2 µg". These will serve as 2.5X Master Mixes for each of the three conditions.

   NOTE: The sterile diluent should be 150 mM NaCl which is essential for DNA/GenJet complex formation. IT IS IMPORTANT THAT THE DNA IS ADDED FIRST AND THE GenJet™ Reagent IS ADDED SECOND TO EACH TUBE.
6. Aliquot 2.5 µg of bacmid into the "1 µg" Master Mix tube.
7. Aliquot 5 µg of bacmid into the "2 µg" Master Mix tube.
8. Briefly vortex the tubes.
9. To the "1 µg" Master Mix tube, add 10 µl of GenJet™ Plus Reagent and IMMEDIATELY VORTEX for 5 seconds.
10. To the "2 µg" Master Mix tube, add 20 µl of GenJet™ Reagent and IMMEDIATELY VORTEX for 5 seconds.
11. Allow the Master Mix tubes to sit in the hood for 10~15 minutes.
12. During the 10~15 minutes incubation period, remove the freshly seeded plates from the incubator. Remove the media from each well, and wash adherent cell monolayer 1X with 2 ml of unsupplemented SF900II media.
13. Add 2 ml of SF900II + gentamicin to each well.
14. After the 10-15 minutes incubation, mix the contents of each Master Mix via gentle pipetting (DO NOT REVORTEX).
15. Add 200 µl of each Master Mix to the appropriate well, and mix by gently rocking the plate(s).
16. Place plate(s) on a level surface at 27° C
18. Harvest supernatants at day 5 post-transfection, for use in high titer stock production.

Storage: This product is stable at 4 °C for 18 months after receipt. This item shipped at ambient temperature