SKU # SL100488 Store at 4 °C

GenJet™ In Vitro DNA Transfection Reagent

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

- ☐ 100 µL
- ☐ 500 µL
- ☐ 1000 µL

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

Introduction

GenJet™ DNA In Vitro Transfection Reagent is a high-performance transfection reagent designed to achieve efficient, reproducible gene delivery with minimal cytotoxicity. GenJet™ is formulated through covalent cross-linking of cationic liposomes with a polymer backbone, a hybridization strategy that confers both exceptional transfection efficiency and markedly reduced cellular toxicity compared to conventional lipid-based systems. The reagent has been validated for gene delivery across a broad panel of established cell lines as well as primary cell cultures.

Procedures for Transfecting Bacmids into Sf9 Cells

This protocol describes the generation of recombinant baculovirus in Sf9 cells through bacmid transfection using a 6-well plate format on a per-well basis. For other culture formats, scale all transfection components proportionally according to the Sf9 cell culture surface area.

1. Harvest and count Sf9 cells, then adjust the cell density to **5×10^5 cells/mL** in serum-free, unsupplemented SF900 II medium.
2. Seed **2 mL** of the cell suspension (**1×10^6 cells/well**) into each well of a sterile 6-well plate.
3. Incubate the plate at **27° C for 30–60 minutes** to allow the Sf9 cells to attach uniformly to the bottom surface of the wells.
4. Add **200 µL** of sterile PBS (without Ca^{2+} and Mg^{2+}) into a sterile 1.5 mL microcentrifuge tube.
5. Add **2.0 µg** of bacmid DNA to the PBS solution and gently pipette up and down 3–4 times to mix thoroughly.
6. Immediately add **6.0 µL** of GenJet™ reagent directly into the diluted bacmid DNA solution. Gently pipette up and down 3–4 times to mix. Avoid vortexing to minimize shearing of the bacmid DNA and disruption of DNA–reagent complex formation.
7. Incubate the transfection mixture at room temperature for **10–15 minutes** to allow stable DNA–reagent complexes to form.
8. Add the entire transfection mixture dropwise to one well of Sf9 cells while gently rocking the plate to ensure even distribution across the cell monolayer.
9. Return the plate to incubation at **27° C**.
10. At **24 hours post-transfection**, carefully replace the medium with **2 mL** of fresh complete SF900 II medium per well.
11. Continue incubation at **27° C** and monitor the cells daily for baculovirus-associated cytopathic effects (CPE), including increased cell diameter, enhanced granularity, reduced proliferation, and decreased viability.
12. Once sufficient CPE is observed, collect the recombinant baculovirus-containing supernatant for downstream amplification, titer determination, or other experimental applications.

Storage: GenJet™ Reagent is stable for 24 months at +4 °C. This item shipped at ambient temperature