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GenJet™ In Vitro DNA Transfection Reagent (Version II)

------- A General Protocol for Transfecting HEK293 and CHO Cells in Suspension

☐ 100 µL
☐ 500 µL
☐ 1000 µL

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

Introduction

GenJet™ In Vitro DNA Transfection Reagent (Ver. II) is an advanced-generation formulation of GenJet™ In Vitro DNA Transfection Reagent incorporating an optimized chemical composition. The reformulated chemistry incorporates an increased density of DNA-condensing moieties relative to the original GenJet™ formulation, resulting in more compact and stable lipoplex formation. This structural enhancement translates to a 3- to 4-fold improvement in DNA delivery efficiency compared to the first-generation reagent. GenJet™ (Ver. II) has been validated for efficient gene delivery across a broad panel of established cell lines as well as primary cell cultures, consistently achieving exceptional transfection efficiency with negligible cytotoxicity.

Important Guidelines for Transfection

- GenJet™ is formulated exclusively for plasmid DNA transfection. It is not intended for siRNA, mRNA, or other nucleic acid formats without prior optimization.
- For protocols covering lentiviral, rAAV, or adenoviral vector production, contact SignaGen at info@signagen.com or visit https://signagen.com.
- Dilute both the plasmid DNA and GenJet™ reagent independently in serum-free DMEM immediately before complex formation. Do **not** use Opti-MEM, as residual serum components can interfere with lipoplex assembly.
- Use endotoxin-free, high-purity plasmid DNA (A_{260}/A_{280} ≥ 1.8; free of phenol, ethanol, and salt contaminants).

Recommended Conditions for Transfection:

The parameters below are optimized for suspension 293 and CHO cells. Scale all reagent volumes proportionally when working at larger culture volumes.

Parameter	Value
Final culture volume	32 mL
Cell number	3 × 10^7 cells
Cell density	1 × 10^6 cells/mL
Plasmid DNA	~20 µg
GenJet™ Reagent	~48 µL
GenJet™ : DNA ratio	2.4 : 1 (v/µg)
Cell viability (required)	> 90%

Procedures for Transfecting Suspension 293 or CHO Cells:

The protocol below is optimized for a 30 mL suspension culture. Scale reagent volumes proportionally for larger volumes.

Day −1 (24 h pre-transfection)

Enumerate cells and seed suspension 293 or CHO cells such that the culture reaches a density of 3 × 10^7 total cells in 30 mL standard culture medium by the following day (~24 h).

Day 0 (Transfection)

Assess cell viability by trypan blue exclusion or an equivalent method. Viability must exceed 90% before proceeding. Adjust the suspension to 1.0 × 10^6 cells/mL in a total volume of 30 mL standard culture medium in an appropriately sized shaker flask.

Important: *Ensure a homogeneous single-cell suspension. If cell aggregates are present, vortex vigorously for 10–30 s immediately before transfection. Cell viability must be >90% to achieve optimal transfection efficiency.*

Lipoplex Preparation

Prepare lipid–DNA complexes as follows:

1. Dilute 20 µg of plasmid DNA in serum-free DMEM to a final volume of 1 mL. Mix by gently pipetting up and down 3-4 times.
2. Separately, dilute 48 µL of GenJet™ Reagent in serum-free DMEM to a final volume of 1 mL. Mix by gently pipetting up and down 3-4 times
3. Add the diluted GenJet™ solution to the diluted DNA solution in a single addition to achieve a total volume of 2 mL. Mix by gently pipetting up and down 3-4 times
4. Incubate the mixture at room temperature for exactly 10 min to allow lipoplex formation.

Important: *Do not use Opti-MEM for dilution; trace serum in Opti-MEM inhibits electrostatic condensation of lipid–DNA complexes.*

Transfection and Culture

– Transfer the entire 2 mL lipoplex mixture into the shaker flask containing the 30 mL suspension culture. Mix gently by swirling.
– Incubate cells at 37° C in a humidified atmosphere containing 8% CO2, on an orbital shaker at 125 rpm.
– Harvest cells (or conditioned medium for secreted proteins) at approximately 48 h post-transfection. Assay for recombinant protein expression by the appropriate method (e.g., western blot, ELISA, flow cytometry).

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