SKU # SL100489 Store at 4 °C

GenJet™ In Vitro DNA Transfection Reagent (Ver. II)

------- A General Protocol for Transfecting Mammalian 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™ 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.

Procedures for Transfecting Mammalian Cells

1. Adherent Cell Transfection

Cell Seeding:

Cells should be seeded 18–24 hours prior to transfection to achieve a monolayer confluence of approximately 80% at the time of transfection. Fresh complete culture medium supplemented with serum and antibiotics should be added to each well 30–60 minutes before initiating the transfection procedure.

Note: *Elevated serum concentrations (>5%) in combination with antibiotics do not typically inhibit transfection efficiency with this reagent. For certain cell types, optimal transfection efficiency is observed under conditions with serum and antibiotics present. Use of complete medium containing both serum and antibiotics is recommended as the default starting condition.*

Table 1. Recommended Reagent and DNA Amounts by Culture Vessel Format

Culture Dish	Culture Medium Volume (mL)	Plasmid DNA (µg)	Serum-Free Diluent Volume (µL)	GenJet™ Reagent Ver. II (µL)
48 well plate	0.5	0.25	30	0.75
12 well plate	0.75	0.75	70	2.25
6-well plate	2.0	1	100	3.0
35 mm dish	2.0	1	100	3.0
60 mm dish	5	2.5	200	7.5
10 cm plate	10	4 – 6	500	12 - 18
T75 flask	10	4 – 6	500	12- 18
15 cm plate	20	10 - 18	1000	30 - 54

Preparation of GenJet™–DNA Complexes and Transfection Procedure

The optimal volumetric ratio of GenJet™ (µL) to DNA (µg) varies by cell type, typically ranging from 2:1 to 3:1. A GenJet™:DNA ratio of 3:1 (µL:µg) is recommended as an initial condition, as this generally yields satisfactory transfection efficiency with negligible cytotoxicity. To ensure optimal complex particle size and colloidal stability, both the DNA and GenJet™ reagent must be diluted in the serum- and antibiotic-free counterpart of the complete culture medium prior to complexation. For example, if cells are maintained in DMEM (high glucose) supplemented with 10% FBS and 1 x penicillin-streptomycin, serum-free DMEM (high glucose) should be used as the diluent.

Standard Transfection Protocol (24-Well Plate Format)

The following protocol is optimized for transfection in 24-well plates. For other culture vessel formats, refer to **Table 1** for scaled reagent volumes. The conditions described below represent a general protocol suitable for the majority of adherent cell lines.

- **Medium Preparation:** Aspirate existing medium from each well and replace with 1.0 mL of fresh complete medium containing serum and antibiotics 30–60 minutes prior to transfection.
- **DNA Dilution:** For each well, add 0.5 µg of plasmid DNA to 50 µL of serum- and antibiotic-free culture medium in a 1.5 mL microcentrifuge tube. Gently mix immediately by pipetting 3–4 times.
- **GenJet™ Addition:** Add 1.5 µL of GenJet™ reagent directly to the diluted DNA solution. Gently mix immediately by pipetting 3–4 times.

  ⚠ ***Critical:*** *Do not use Opti-MEM as the diluent for either the DNA or GenJet™ reagent. Opti-MEM disrupts complex formation and significantly reduces transfection efficiency.*
- **Complex Formation:** Incubate the transfection mix at room temperature for 15–20 minutes to allow GenJet™–DNA complexes to form.

  ⚠ ***Critical:*** *Do not exceed 20 minutes of incubation. Prolonged incubation leads to aggregate formation and reduced transfection efficiency.*
- **Transfection:** Add the entire volume of GenJet™–DNA complex dropwise onto the cells in each well. Gently swirl the plate to ensure uniform distribution of the complex across the cell monolayer. Return the plate to the 37 ºC / 5% CO_2 incubator immediately.
- **Medium Replacement:** Approximately 18 hours post-transfection, aspirate the complex-containing medium and replace with fresh complete medium supplemented with serum and antibiotics. For cytotoxicity-sensitive cell types, medium replacement should be performed 5 hours post-transfection to minimize potential cytotoxic effects.
- **Assessment of Transfection Efficiency:** Transfection efficiency should be evaluated 24–48 hours post-transfection

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using an appropriate method, such as fluorescence microscopy, flow cytometry, or luminescence-based reporter assay, depending on the nature of the transgene and the sensitivity required. In most cell types, assessment at 48 hours post-transfection yields higher transgene expression levels compared to 24 hours, likely reflecting the time required for complete nuclear import, transcription, and accumulation of detectable reporter protein. Assessment at 48 hours post-transfection is therefore recommended as the standard evaluation time point.

2. Suspension Cell Transfection (6-Well Plate Format)

The following protocol is adapted for transfection of suspension cells in a 6-well plate format. A GenJet™ (µL):DNA (µg) ratio of 3:1 is recommended as the initial condition. All transfection conditions, including DNA amount, GenJet™ volume, and diluent volume, can be scaled proportionally to the tissue culture surface area of any culture vessel format.

Step 1 — Cell Preparation: Count cells and seed at the appropriate density in complete culture medium 18–24 hours prior to transfection to ensure cells are in the exponential growth phase at the time of transfection. Optimal cell viability (>90%) is critical for achieving high transfection efficiency.

Step 2 — Medium Preparation: 30–60 minutes prior to transfection, centrifuge the suspension cell culture at 200–300 x g for 5 minutes, aspirate the spent medium, and resuspend the cell pellet in 2.0 mL of fresh complete medium supplemented with serum and antibiotics per well.

Step 3 — DNA Dilution: For each well, add **2.0 µg** of plasmid DNA to **200 µL** of serum- and antibiotic-free culture medium in a 1.5 mL microcentrifuge tube. Gently mix immediately by pipetting 3–4 times.

Step 4 — GenJet™ Addition: Add **6.0 µL** of GenJet™ reagent directly to the diluted DNA solution (Step 3). Gently mix immediately by pipetting 3–4 times.

⚠ ***Critical:*** *Do not use Opti-MEM as the diluent for either the DNA or GenJet™ reagent. Opti-MEM disrupts GenJet™–DNA complex formation and significantly reduces transfection efficiency.*

Step 5 — Complex Formation: Incubate the transfection mix at room temperature for 15–20 minutes to allow GenJet™–DNA complexes to form.

⚠ ***Critical:*** *Do not exceed 20 minutes of incubation. Prolonged incubation leads to aggregate formation and reduced transfection efficiency.*

Step 6 — Transfection: Add the entire volume of GenJet™–DNA complex dropwise to the suspension cell culture. Mix gently by pipetting 2–3 times to ensure uniform distribution of the complex throughout the cell suspension. Do not vortex the cells after complex addition.

Step 7 — Post-Transfection Medium Replacement: Approximately 18 hours post-transfection, centrifuge the culture at 200–300 x g for 5 minutes, aspirate the complex-containing medium, and resuspend the cell pellet in **2.0 mL** of fresh complete medium supplemented with serum and antibiotics. For cytotoxicity-sensitive suspension cell types, medium replacement should be performed 5 hours post-transfection to minimize potential cytotoxic effects.

Step 8 — Assessment of Transfection Efficiency: Transfection efficiency should be evaluated 24–48 hours post-transfection using an appropriate method, such as

fluorescence microscopy, flow cytometry, or luminescence-based reporter assay, depending on the nature of the transgene and the sensitivity required. In most cell types, assessment at 48 hours post-transfection yields higher transgene expression levels compared to 24 hours, likely reflecting the time required for complete nuclear import, transcription, and accumulation of detectable reporter protein. Assessment at 48 hours post-transfection is therefore recommended as the standard evaluation time point.

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