Cat # SL100488 Store at 4 °C

GenJet™ DNA In Vitro Transfection Reagent

----- A Protocol for Transfections of Mammalian Cell

- ☐ 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 Tranfection Reagent is a powerful transfection Reagent that ensures effective and reproducible transfection with extremly low toxicity. GenJet™ is formulated by covalently cross-linking cationic liposome with polymer, giving rise to exceptional transfection efficiency and extreme low toxicity. GenJet™ 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™ 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
- Extreme low cytotoxicity
- Efficient transfection with or without serum
- High levels of recombinant protein production
- Simple, robust transfection procedure
- Effectively transfects both adherent and suspension cell cultures

Procedures for Transfecting Mammalian Cells:

1. For Adherent Cells

Cell Seeding (see table 1):

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal 50-60% confluency at the time of transfection. Serum-free DMEM medium is changed to replace complete serum-containing culture medium 30 minutes before transfection.

Note: High serum levels (>5%) have a moderate inhibitory effect on GenJet™-mediated transfections. Maximal transfection efficiencies are observed in the absence of serum. Depending upon the cell type, the presence of serum <5% may sometimes improve the overall levels of recombinant protein expression.

Table 1. A guideline for seeding adherent cells prior to transfection in different culture formats.

Culture Dishes	Surface Area (cm^2)	Number of Cells to Seed
T175 Flask	175	0.7–1.4 x 10^7
T75 Flask	75	3.0–6.0 x 10^6
100 mm Dish	58	2.2–4.4 x 10^6
60 mm Dish	21	0.9–1.8 x 10^6
35 mm Dish	9.6	3.5–7.0 x 10^5
6-well Plate	9.6	4.0–8.0 x 10^5
12-well Plate	3.5	1.5–3.0 x 10^5
24-well Plate	1.9	0.8–1.6 x 10^5
48-well Plate	1.0	4.0–8.0 x 10^4
96-well Plate	0.3	1.2–2.4 x 10^4

Preparation of GenJet™-DNA Complex and Transfection Procedures

The optimal ratio of GenJet™/DNA is of 3/1 (3 µL of GenJet™ Reagent is used per 1 µg of plasmid DNA). We recommend using serum-free DMEM with High Glucose to dilute DNA and GenJet™ Reagent to ensure the optimal size of complex particles.

The following protocol is given for transfection in 24-well plates, refer to table 2 for transfection in other culture formats. The optimal transfection conditions for a majority of adherent cell lines, as well as a general starting point for optimization are given in the standard protocol described below.

- For each well, dilute 1 µg of DNA into 50 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.
- For each well, dilute 3 µl of GenJet™ solution into 50 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.
- Add the 50 µl GenJet™ solution to the 50 µl DNA solution all at once. (**Imortant: do not mix the solutions in the reverse order !**)
- Vortex- mix the solution immediately and spin down briefly to bring drops to the bottom of the tube.
- Incubate for 10 minutes at room temperature.
- Add the 100 µl GenJet™/ DNA mixture drop-wise onto the medium in each well and homogenize the mixture by gently swirling the plate.
- For maximal transfection efficiency, change the medium to complete serum containing medium 4~5 hours post addition of GenJet™/DNA complex.
- Check transfection efficiency24 to 48 hours post transfection.

2. For Suspension Cells

The following protocol is given for transfection in 6-well plate. The protocol can be scalded up or down according to culture volume.

Cell Seeding: Suspension cells are typically seeded the day of the transfection at a density of 0.5–1.0 x 10^6 cells per ml of culture. For optimal transfection conditions with GenJet™, seed the number of cells adapted to the culture vessel format according to table 3.

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Table 2. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Culture Volume (mL)	Plasmid DNA (µg)	Diluent (mL)	GenJet™ Reagent (µL)
6-well plate	3	2 – 4	0.3	6 – 12
35 mm dish	3	2 – 4	0.3	6 – 12
60 mm dish	5	6 – 12	0.5	18 – 36
100 mm dish	10	12 – 24	1.0	36 – 72
T75 flask	15	18 – 36	1.5	54 – 108
250-mL flask	50	50 – 100	2.5	150 – 300

Table 3. Recommended number of suspension cells to seed.

Culture Dish	Number of Cells
96-well plate	2×10^4 – 5×10^4
48-well plate	5×10^4 – 1×10^5
24-well plate	1×10^5 – 2×10^5
6-well plate	2×10^5 - 5×10^5
35 mm dish	5×10^5 – 2×10^6
60 mm dish	2×10^6 – 5×10^6
100 mm dish	5×10^6 – 1×10^7

GenJet™/DNA Complex Preparationand Transfection Procedures:
The optimal ratio of GenJet™/DNA is of 3/1 (3 µL of GenJet™ Reagent is used per 1 µg of plasmid DNA). We recommend using serum-free DMEM with High Glucose to dilute DNA and GenJet™ Reagent to ensure the optimal size of complex particles.

The following protocol is given for transfection in 6-well plates.

- For each well, dilute 2 – 3 µg of DNA into 100 µl of serum-free DMEM with high glucose. Vortex gently and spin down briefly.
- For each well, dilute 6 – 9 µl of GenJet™ solution into 100 µl of serum-free DMEM with high glucose. Vortex gently and spin down briefly.
- Add the 100 µl GenJet™ solution to the 100 µl DNA solution all at once **(important: do not mix the solutions in the reverse order)**
- Vortex- mix the solution immediately and spin down briefly to bring drops to the bottom of the tube.
- Incubate for 10 minutes at room temperature.
- Add the 200 µl GenJet™/ DNA mixture drop- wise onto the serum containing medium in each well, homogenize the mixture by gently swirling the plate.
- Incubate at 37° C and 5% CO2 in a humidified atmosphere.
- Transfection experiments are usually stopped after 24 to 48 hours and gene activity assessed. Cells growing in suspension are collected by centrifugation at 800xg and then resuspended in the desired medium or buffer.

Storage: GenJet™ DAN In Vitro Transfection Reagent is stable for up to 18 months at 4 °C. This item shipped at ambient temperature