Cat # SL100488 Store at 4 °C

GenJet™ In Vitro DNA Transfection Reagent

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

- ☐ 100 µl
- ☐ 500 µl
- ☐ 1000 µl

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Introduction:

GenJet™ DNA In Vitro Tranfection Reagent is a powerful transfection Reagent that ensures effective and reproducible transfection without cytotoxicity. 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.

Features:

- No cytotoxicity for most of tumor cell lines and primary cells
- Exceptional transfection efficiency of a broad range of cell types
- Efficient transfection with or without serum
- High levels of recombinant protein production
- Simple, robust transfection procedure

Procedures for Transfecting Mammalian Cells

1. For Adherent Cells

Cell Seeding:

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal 70~80% confluency at time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30~60 minutes before transfection.

Note: High serum levels (>5%) with antibiotics usually do not have inhibitory effect on transfection efficiency. For some specific cells, maximal transfection efficiencies are observed in the presence of serum and antibiotics. We recommend using complete serum/antibiotics-containing medium initially.

Table 1. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Transfection Volume (ml)	Plasmid DNA (µg)	Diluent Volume (mL)	GenJet Reagent (µL)
48 well plate	0.3	0.25	2 x 0.015	0.75
12 well plate	0.75	0.75	2 x 0.038	2.25
6-well plate	1.0	1	2 x 0.05	3.0
35 mm dish	1.0	1	2 x 0.05	3.0
60 mm dish	2.8	3	2 x 0.10	9
10 cm dish	5.0	5	2 x 0.25	15
T75 flask	8.0	9 - 18	2 x 0.40	27 - 54
250 ml flask	18	25 - 50	2 x 0.8	75 - 150

Preparation of GenJet™-DNA Complex and Transfection Procedures

For different cell types, the optimal ratio of GenJet™ (µL): DNA (µg) varies from 2:1 to 3:1. We recommend the GenJet™ (µL):DNA (µg) ratio of 3:1 as a starting point which usually gives satisfactory transfection efficiency with invisible cytotoxicity. To ensure the optimal size of complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and GenJet™ Reagent.

The following protocol is given for transfection in 24-well plates, refer to **Table 1** for transfection in other culture formats. The optimal transfection conditions for a majority of adherent cell lines are given in the standard protocol described below.

- For each well, add 0.5 ml of complete medium with serum and antibiotics freshly 30~60 minutes before transfection.
- For each well, dilute 0.5 µg of DNA into 25 µl of serum-free DMEM with High Glucose. Pipette up and down 3~4 times to mix.
- For each well, dilute 1.5 µl of GenJet™ reagent into 25 µl of serum-free DMEM with High Glucose. Pipette up and down 3~4 times to mix.
  Note: Never use Opti-MEM to dilute GenJet™ reagent and DNA, it will disrupt transfection complex.
- Add the diluted GenJet™ Reagent immediately to the diluted DNA solution all at once. **(Important: do not mix the solutions in the reverse order !)**
- Immediately pipette up and down 3~4 times to mix or vortex briefly to mix followed by incubation for ~15 min at room temperature to allow GenJet™/DNA complexes to form.
  Note: Never keep the GenJet™/DNA complex longer than 30 minutes
- Add the 50 µl GenJet™/ DNA complex drop-wise onto the medium in each well and homogenize the mixture by gently swirling the plate.
- Remove GenJet™/DNA complex-containing medium and replace with complete serum/antibiotics containing medium 12~18 hours post transfection. **For sensitive cells, to lower cytotoxicity, remove GenJet™/DNA complex and replace with complete medium 5 hours after transfection.**
- Check transfection efficiency 24 to 48 hours post transfection.

Storage: GenJet™ Reagent is stable for 12 months at +4 °C. This item shipped at ambient temperature

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GenJet™ DNA In Vitro Transfection Reagent

----- A Protocol for Transfections of Mammalian Cell (cont'd)

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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 2**.

Table 2. Recommended Number of Suspension Cells to Seed

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

GenJet™/DNA Complex Preparation and Transfection Procedures

For different cell types, the optimal ratio of GenJet™ (µL):DNA (µg) varies from 2:1 to 3:1. We recommend the GenJet™ (µL):DNA (µg) ratio of 3:1 as a starting point which usually gives satisfactory transfection efficiency with invisible cytotoxicity. To ensure the optimal size of complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and GenJet™ reagent.

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

- For each well, dilute 2 µg of DNA into 50 µl of DMEM Serum-free Medium with High Glucose. Vortex gently and spin down briefly.
- For each well, dilute 6 µl of GenJet™ reagent into 50 µl of DMEM Serum-free Medium with High Glucose. Vortex gently and spin down briefly.
- Add the 50 µl GenJet™ solution immediately to the 50 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 15~20 minutes at room temperature.
- Add the 100 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% CO_2 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 800 x g and then resuspended in the desired medium or buffer.