Cat # SL100499 Store at 4 °C

GenJet™ Plus In Vitro DNA Transfection Reagent

----- A General Protocol for Transfecting 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™ Plus DNA In Vitro Tranfection Reagent is enhanced version of GenJet™ Plus DNA In Vitro Transfection Reagent. Compared with its previous version, GenJet™ Plus was formulated by refined chemistry with addition of an enhancer and was confirmed to be more powerful in delivering DNA to various established cell lines as well as primary cells.

Important Guidelines for Transfection:

- GenJet™ Plus reagent was formulated for DNA transfection ONLY! The following standard protocol is for transfecting mammalian cells. To request protocol for lentivirus production and insect cells transfection, please email us at info@signagen.com
- For better efficiency, choosing a correct protocol is essential. We strongly encourage to use "General Protocol" first. If the "General Protocol" fails to give satisfactory result (e.g., less than 10%), try the "Advanced Protocol" in the back page
- For high efficiency and lower toxicity, transfect cells at high density. 70~80% confluency is highly recommended
- To lower cytotoxicity, transfect cells in presence of serum (10%) and antibiotics

Part I. A General Protocol for Transfecting Adherent Cells

Step I. 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 the 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. We recommend using complete serum/antibiotics-containing medium as a starting point. For maximal efficiency and lower cytotoxicity, perform transfection on cells with high density. We recommend transfecting on cells with ~90% confluency.

Step II. Preparation of GenJet™ Plus-DNA Complex and Transfection Procedures:

For different cell types, the optimal ratio of GenJet™ Plus (µL):DNA (µg) is around 3:1. We recommend the GenJet™ Plus (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 GenJet™/DNA complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and GenJet™ Plus 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, as well as a general starting point for optimization 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. Vortex gently or pipette up and down 3~4 times to mix.
- For each well, dilute 1.5 µl of GenJet™ Plus reagent into 25 µl of serum-free DMEM with High Glucose. Gently pipette up and down 3~4 times to mix.

Note: Never use Opti-MEM to dilute GenJet™ Plus reagent and DNA, it will disrupt transfection complex.

- Add the diluted GenJet™ Plus 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 or vortex briefly to mix.
- Incubate for ~10 minutes at room temperature to allow GenJet™/DNA complexes to form.

Note: Never keep the GenJet™/DNA complex longer than 20 minutes.

- Add the 50 µl GenJet™/ DNA mixture 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 fresh 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.

Table 1. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Culture Medium (ml)	Plasmid DNA (µg)	Diluent Volume (mL)	GenJet™ Plus Reagent (µL)
48 well plate	0.2	0.25	2 x 0.0125	0.75
12-well plate	0.75	0.75	2 x 0.038	2.25
6-well plate	1	1	2 x 0.05	3
35 mm dish	1	1	2 x 0.05	3
60 mm dish	2.8	2.5	2 x 0.1	7.5
10 cm dish	5	5	2 x 0.25	15
T75 flask	8	9 - 18	2 x 0.40	27 - 54
250 ml flask	18	25 - 50	2 x 0.8	75 - 150

Storage: GenJet™ Plus Reagent is stable for up to 12 months at +4 °C after receipt

Cat # SL100499 Store at 4 °C

GenJet™ Plus In Vitro DNA Transfection Reagent

----- An Advanced Protocol for Transfecting Hard-to-Transfect Cells

- ☐ 100 µl
- ☐ 500 µl
- ☐ 1000 µl

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Part II. Advanced Protocol for Transfecting Hard-To-Transfect Mammalian Cells

Important: The advanced protocol for hard-to-transfect cells is provided only if general protocol gives less than 10% efficiency. **For some primary cells which cannot be trypsinized (like primary neurons), go directly to Step II, skip trypsinization and incubate freshly prepared primary cell pellet with transfection complex.**

Step I. Culturing of Cells Before Transfection:

Cells should be plated at least 24 hours prior to transfection so that the monolayer cell density reaches to the optimal 95~100% confluency at the day of transfection.

Table 2. A Guideline for Optimal Cell Number Per Well in Different Culture Formats

Culture Dishes	Surface Area (cm^2)	Optimal Cell Number
T75 Flask	75	9.6×10^6
100 mm Dish	58	7.3×10^6
60 mm Dish	21	2.7×10^6
35 mm Dish	9.6	1.0×10^6
6-well Plate	9.6	1.0×10^6
12-well Plate	3.5	0.44×10^6
24-well Plate	1.9	0.24×10^6
48-well Plate	1.0	0.11×10^6
96-well Plate	0.3	0.31×10^5

Table 3. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Transfection Complex Volume (ml)	Plasmid DNA (µg)	GenJet™ Plus Reagent (µL)
96-well	0.02	0.2	0.8
48-well	0.04	0.5	2
24-well	0.1	1	4
6-well	0.2	2	8
35 mm dish	0.2	2	8
60 mm dish	0.5	5	20
10 cm dish	1.0	8	32
T75 flask	1.5	36	144
250 ml flask	2.5	100	400

Step II. Preparation of Cells in Suspension:

The following protocol is given for transfecting hard-to-transfect cells in 6-well plates, refer to **Table 2** for optimal cell number per well per culture vessels' surface area. The optimal transfection conditions are given in the standard protocol described below.

- Detach the cells with trypsin/EDTA and stop the trypsinization with complete culture medium.
  Note: Cells that are difficult to detach may be placed at 37 °C for 5-15 min to facilitate detachment
- Take an aliquot of trypsinized cell suspension and count the cells to determine the cell density.
- Centrifuge the required ~1.0×10^6 cells per well for 6-well plate at 150xg at room temperature for 10 min.
- Use fine tip pipette to remove supernatant **completely** so that no residual medium covers the cell pellet.

Step III. Preparation and application of Transfection Complex:

For most of mammalianells, the optimal ratio of GenJet™ Plus (µL):DNA (µg) is 4:1. To ensure the optimal size of complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and GenJet™ Plus Reagent.

The following protocol is given for transfection in 6-well plates, refer to **Table 3** for transfection in other culture formats.

- For each well of 6-well plate, dilute 2 µg of DNA into 100 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly to bring drops to bottom of the tube.
- For each well of 6-well plate, dilute 8 µl of GenJet™ Plus reagent into 100 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.
- Add the diluted GenJet™ Plus Reagent immediately to the diluted DNA solution all at once. **(Important: do not mix the solutions in the reverse order !)**
- Vortex-mix the solution immediately or pipette up and down 3~4 times to mix followed by incubation for ~15 minutes at room temperature to allow transfection complex to form.
  Note: Never keep the transfection complexes longer than 20 minutes
- **Gently** resuspend the cell pellet prepared from **Step II** immediately in the 200 µl transfection complex and incubate at 37 °C for 20 minutes.
- At the end of incubation, add 2.0 ml of pre-warmed fresh complete cell growth medium to cells and plate onto one well of a 6-well plate. Incubate at 37 °C with 5% CO_2.
- Remove transfection complex containing medium **gently** and refill with complete culture medium 8~12 hours after plating.
- Check transfection efficiency 24 to 48 hours post transfection.