

GenJet™ Plus In Vitro DNA Transfection Reagent

----- A Protocol for Transfections of Insect Cells

- 100 µl
- 500 µl
- 1000 µl



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

Introduction:

Based on our innovative polymer synthesis technology, GenJet™ Plus DNA In Vitro Transfection Reagent was formulated to be a powerful transfection Reagent that ensures effective and reproducible transfection with less cytotoxicity. GenJet™ Plus was shown to deliver genes to various established cell lines as well as primary cells. The following protocol is given for transfecting insect cells.

PART 1. Transient transfection protocol for adherent insect cells

The protocol is adapted for standard cell lines such as Sf9, Sf21.

1.1 Cell seeding

The cell density is a critical parameter for efficient insect cells transfection. In order to achieve optimal transfection efficiency with GenJet™ Plus reagent, we recommend preparing a cell suspension at 1.25×10^6 cells/mL. The volume to dispense per well is shown in **Table 1**.

Table 1. Recommended volume of cell suspension to seed 2 h before transfection

Culture Vessel	Volume of Cell Suspension to Add per Well	Corresponding Number of Cells per Well
96-well	80 µL	1×10^5
24-well	400 µL	5×10^5
6-well/35 mm	1.6 mL	2×10^6
10 cm	4 mL	5×10^6

Table 2. Amount of DNA and volumes of reagent to be used for transfection of most insect cells according to the cell culture formats

Culture vessel	Amount of DNA (µg)	Volume of GenJet™ 1:2 ratio (µL)	Volume of dilution buffer for DNA and GenJet™ Plus (µL)	Total volume of Complexes added per well (µL)	Volume of medium to add after 5 h
96-well	0.53	1	10	20	120 µL
24-well	2	4	50	100	500 µL
6-well	6	12	100	200	2 mL
10 cm	17	34	500	1000	8 mL

1.2 Transfection Procedures

We recommend using a 1:2 ratio DNA to GenJet™ reagent (w/v). Per well in 6 well plates:

- Seed 1.6 mL of a cell suspension at 1.25×10^6 cells/mL.
- Allow cells to attach for 2 h in the incubator.
- Dilute 6 µg DNA into 100 µL insect cell culture medium (without serum), mix by brief vortexing.
- Dilute 12 µL GenJet™ reagent into 100 µL insect cell culture medium (without serum), mix by brief vortexing.
- Add the diluted 112 µL GenJet™ reagent to 100 µL diluted DNA followed by vortexing for 10 s to mix and incubating for 15 min at RT.
- Add 200 µL GenJet™/DNA complexes dropwise to the cells.
- Gently agitate the plates and return the plates to the incubator for 5 h.
- Add 2 mL of medium with serum (and antibiotics /antimycotics if required).
- Proceed with protein purification after 72 h or as required.

For other culture formats, apply the volumes and amounts indicated in **Table 2 to the 6-well plates protocol above.**

Storage: This product is stable at 4 °C ~ room temperature for 18 months after receipt. This item shipped at ambient temperature

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----- A Protocol for Transfections of Insect Cells (Cont'd)

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PART 2. Transfection protocol for insect cells grown in suspension

The protocol is adapted for standard cell lines such as Sf9, Sf21.

2.1 Cell seeding

The cell density is a critical parameter for efficient insect cells transfection. In order to achieve optimal transfection efficiency with GenJet™ Plus reagent, we recommend preparing a cell suspension at 10^6 cells/mL in synthetic medium and antibiotics if needed. Keep under agitation at 50 to 100 rpm.

2.2 Transfection procedure in suspension

We recommend using a 1:2 ratio DNA to GenJet™ Plus reagent (w/v). Per mL of culture, use 2 µg of DNA and 4 µL of GenJet™ Plus reagent.

Per mL of cell culture volume:

- Seed the required volume of cell suspension at 10^6 cells/mL. Keep under agitation.
- Dilute the DNA in synthetic medium (without serum) as indicated in **Table 3**, mix by vortexing briefly.
- Dilute GenJet™ reagent in synthetic culture medium as indicated in Table 3, mix by vortexing briefly.
- Add diluted GenJet™ reagent to the diluted DNA, vortex for 10 s and incubate for 15 min at RT.

- Add the GenJet™ /DNA complexes to the cells.
- Incubate for 24 to 72 h under agitation in standard cell culture conditions.
- Proceed with protein purification as required.

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Table 3. Volume of complexes to prepare according to the chosen volume of culture

Volume of cell suspension	Size of cell culture vessel	Volume of cell culture medium for dilution of DNA and GenJet™ Plus	Volume of complexes added to the cells
3 mL	50 mL Tube	100 µL	200 µL
25 mL	250 mL Erlenmeyer	1 mL	2 mL
50 mL	250 mL Erlenmeyer	2 mL	4 mL
1 L	Bioreactor	40 mL	80 mL