



# GenJet™ DNA In Vitro Transfection Reagent



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

- 100 µl
- 500 µl
- 1000 µl

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

## 2. For Suspension Cells

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

**Cell Seeding:** Suspension cells are typically seeded the day of the transfection at a density of  $0.5 \sim 1.0 \times 10^6$  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	$2 \times 10^4 - 5 \times 10^4$
48-well plate	$5 \times 10^4 - 1 \times 10^5$
24-well plate	$1 \times 10^5 - 2 \times 10^5$
6-well plate	$2 \times 10^5 - 5 \times 10^5$
35 mm dish	$5 \times 10^5 - 2 \times 10^6$
60 mm dish	$2 \times 10^6 - 5 \times 10^6$
100 mm dish	$5 \times 10^6 - 1 \times 10^7$

## 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<sub>2</sub> 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.