## Cat # SL100488 Store at 4 °C

## GenJet™ DNA In Vitro Transfection Reagent

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



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

100 μl

500 μl

## 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  $0.5 \sim 1.0 \times 10^6$  cells per ml of culture. For optimal transfection conditions with GenJet<sup>TM</sup>, 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 <sup>4</sup> - 5 x 10 <sup>4</sup>
48-well plate	$5 \times 10^4 - 1 \times 10^5$
24-well plate	1 x 10 <sup>5</sup> - 2 x 10 <sup>5</sup>
6-well plate	2 x 10 <sup>5</sup> - 5 x 10 <sup>5</sup>
35 mm dish	5 x 10 <sup>5</sup> - 2 x 10 <sup>6</sup>
60 mm dish	2 x 10 <sup>6</sup> - 5 x 10 <sup>6</sup>
100 mm dish	5 x 10 <sup>6</sup> - 1 x 10 <sup>7</sup>

## GenJet™/DNA Complex Preparation and Transfection Procedures

For different cell types, the optimal ratio of GenJet<sup>™</sup> (µL):DNA (µg) varies from 2:1 to 3:1. We recommend the GenJet<sup>™</sup> (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<sup>™</sup> reagent.

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

- For each well, dilute 2  $\mu g$  of DNA into 50  $\mu l$  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  $800 \times g$  and then resuspended in the desired medium or buffer.