Cat # SL100488 Store at 4 °C

GenJet™ DNA In Vitro Transfection Reagent

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

- ☐ 100 µl
- ☐ 500 µl
- ☐ 1000 µl

SignaGen® Laboratories
10075 Tyler Place, Suite 19
Ijamsville, MD 21754
FAX. 301-560-4919
TEL. 301-330-5966
Toll Free. 1-(866)-918-6812
Email: info@signagen.com
Web: www.signagen.com

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.