

GenJet™ In Vitro DNA Transfection Reagent for MCF-7 Cells (Ver. II)

----- A Protocol for Transfecting MCF-7 Cells

- 100 µl
- 500 µl
- 1000 µl



15875 Gaither Drive
Gaithersburg, MD 20877
FAX. 301-560-4919
TEL. 301-330-5966
Toll Free. 1-(866)-918-6812
Email: info@signagen.com
Web: www.signagen.com

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

Introduction:

GenJet™ In Vitro DNA Transfection Reagent (Ver. II) is upgraded version of GenJet™ In Vitro DNA Transfection Reagent. With a new chemistry, more DNA condensing groups were released in the new version compared with old version GenJet™, leading to 3~4 times more efficient in DNA delivery. GenJet™ (Ver. II) for MCF-7 cells was Pre-optimized and conditioned for transfecting MCF-7 cells.

Procedures for Transfecting MCF-7 Cells:

Step A. Cell Seeding (see Table 1):

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal ~90% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well ~60 minutes before transfection.

Table 1. A Guideline for Seeding Adherent Cells Prior to Transfection in Different Culture Formats

| Culture Dishes | Surface Area (cm ²) | Number of Cells to Seed |
|----------------|---------------------------------|-----------------------------|
| T75 Flask | 75 | 3.0 – 6.0 × 10 ⁶ |
| 100 mm Dish | 58 | 2.2 – 4.4 × 10 ⁶ |
| 60 mm Dish | 21 | 0.9 – 1.8 × 10 ⁶ |
| 35 mm Dish | 9.6 | 3.5 – 7.0 × 10 ⁵ |
| 6-well Plate | 9.6 | 4.0 – 8.0 × 10 ⁵ |
| 12-well Plate | 3.5 | 1.5 – 3.0 × 10 ⁵ |
| 24-well Plate | 1.9 | 0.8 – 1.6 × 10 ⁵ |
| 48-well Plate | 1.0 | 4.0 – 8.0 × 10 ⁴ |
| 96-well Plate | 0.3 | 1.2 – 2.4 × 10 ⁴ |

Table 2. Recommended Amounts for Different Culture Vessel Formats

| Culture Dish | Transfection Volume (ml) | Plasmid DNA (µg) | Diluent Volume (mL) | GenJet™ Reagent (µL) |
|--------------|--------------------------|------------------|---------------------|----------------------|
| 96-well | 0.2 | 0.2 | 2 × 0.01 | 0.6 |
| 48-well | 0.3 | 0.5 | 2 × 0.02 | 1 |
| 24-well | 0.5 | 1.0 | 2 × 0.05 | 3 |
| 6-well | 1.2 | 2 | 2 × 0.1 | 6 |
| 35 mm dish | 1.5 | 2 | 2 × 0.1 | 6 |
| 60 mm dish | 3 | 5 | 2 × 0.25 | 15 |
| 10 cm dish | 6 | 7 - 8 | 2 × 0.5 | 21 - 24 |
| T75 flask | 10 | 18 - 36 | 2 × 0.75 | 54 - 108 |
| 250 ml flask | 20 | 50 - 100 | 2 × 1.25 | 150 - 300 |

Step B. Preparation of GenJet™-DNA Complex and Transfection Procedures

For MCF-7 cells, the optimal ratio of GenJet™ (µL):DNA (µg) is 3:1. 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 24-well plates, refer to **Table 2** for transfection in other culture formats. The optimal transfection conditions For MCF-7 cells are given in the standard protocol described below.

- For each well, add 0.5 ml of complete medium with serum and antibiotics freshly ~60 minutes before transfection.
- For each well, dilute 1 µg of DNA into 50 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, dilute 3 µl of GenJet™ reagent (Ver. II) into 50 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.
- Add the diluted GenJet™ 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 and spin down briefly to bring drops to bottom of the tube followed by incubation of 15 minutes at room temperature to allow GenJet™-DNA complexes to form.
- Note:** Never keep the DNA/GenJet™ complex longer than 20 minutes
- Add the 100 µl GenJet™/ DNA complex drop-wise onto the medium in each well and homogenize the mixture by gently swirling the plate.
- Remove DNA/GenJet™ complex-containing medium and replace with fresh complete serum/antibiotics containing medium 12~18 hours post transfection.
- Check transfection efficiency 24 to 48 hours post transfection.

Storage: GenJet™ DNA In Vitro Transfection Reagent is stable for up to 12 months at +4 °C. This item shipped at ambient temperature