Cat # SL100489-LNCAP Store at 4 °C

# GenJet™ In Vitro DNA Transfection Reagent for LNCaP Cells (Ver. II)

----- A Protocol for Transfecting LNCaP Cells

- ☐ 100 µl
- ☐ 500 µl
- ☐ 1000 µl

SignaGen® Laboratories
5260 Westview Dr, Suite 100
Frederick MD 21703
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 Tranfection Reagent (Ver. II) is upgraded version of GenJet™ In Vitro DNA Tranfection Reagent. With a new chemistry, more DNA condensing groups were released in the new version compared with old version GenJet™, leading to 3~20 times more efficient in DNA delivery. GenJet™ (Ver. II) for LNCaP cells was pre-optimized and conditioned for transfecting LNCaP cells.

## Procedures for Transfecting LNCaP Cells:

### Step I. 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 ~80% confluency at the time of transfection. Complete culture medium with serum and anti-biotics 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 | $3.0 – 6.0 \times 10^6$ |
| 100 mm Dish | 58 | $2.2 – 4.4 \times 10^6$ |
| 60 mm Dish | 21 | $0.9 – 1.8 \times 10^6$ |
| 35 mm Dish | 9.6 | $3.5 – 7.0 \times 10^5$ |
| 6-well Plate | 9.6 | $4.0 – 8.0 \times 10^5$ |
| 12-well Plate | 3.5 | $1.5 – 3.0 \times 10^5$ |
| 24-well Plate | 1.9 | $0.8 – 1.6 \times 10^5$ |
| 48-well Plate | 1.0 | $4.0 – 8.0 \times 10^4$ |
| 96-well Plate | 0.3 | $1.2 – 2.4 \times 10^4$ |

**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 x 0.01 | 0.6 |
| 48-well | 0.3 | 0.5 | 2 x 0.02 | 1.5 |
| 24-well | 0.5 | 1.0 | 2 x 0.05 | 3 |
| 6-well | 1.0 | 2 | 2 x 0.1 | 6 |
| 35 mm dish | 1.0 | 2 | 2 x 0.1 | 6 |
| 60 mm dish | 3 | 4 | 2 x 0.25 | 12 |
| 10 cm dish | 5 | 7 - 8 | 2 x 0.5 | 21 - 24 |
| T75 flask | 6 | 10 - 13 | 2 x 0.5 | 30 - 39 |
| 250 ml flask | 20 | 28 - 50 | 2 x 1.25 | 84 - 150 |

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

**For LNCaP 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 LNCaP 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.
  **Note:** Never use Opti-MEM to dilute GenJet™ reagent and DNA, it may disrupt transfection complex.
- Add the diluted GenJet™ Reagent immediately to the diluted DNA solution all at once. **(Important: do not mix the solutions in the reverse order !)**
- Immediately pipette up and down 3~4 times to mix followed by incubation for ~15 minutes at room temperature to allow GenJet™-DNA complexes to form.
  **Note:** Never keep the DNA/GenJet™ complex longer than 30 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 ~5 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

© 2010 SignaGen Laboratories