

GenJet™ In Vitro DNA Transfection Reagent for BHK-21 Cells (Ver. II)

----- A Protocol for Transfecting BHK-21 Cells

- 100 µl
 500 µl
 1000 µl



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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 3~20 times more efficient in DNA delivery. GenJet™ (Ver. II) for BHK-21 cells was pre-optimized and conditioned for transfecting BHK-21 cells.

Procedures for Transfecting BHK-21 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 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 | 2 × 0.5 | 21 |
| T75 flask | 10 | 18 | 2 × 0.75 | 54 |
| 250 ml flask | 20 | 50 | 2 × 1.25 | 150 |

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

For BHK-21 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 BHK-21 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 !)**
- Immediately pipette up and down 3~4 times or vortex briefly to mix followed by incubation for 15 minutes at room temperature to allow GenJet™-DNA es complex 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