Cat # SL100489-CACO Store at 4 °C

GenJet™ In Vitro DNA Transfection Reagent for Caco-2 Cells (Ver. II)

----- A General Protocol for Transfecting Caco-2 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 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 Caco-2 cells was pre-optimized and conditioned for transfecting Caco-2 cells.

Important Guidelines for Transfection:

- GenJet™ reagent was formulated for DNA transfection ONLY! The following standard protocol is for transfecting Caco-2 cells.
- For better efficiency, choosing a correct protocol is essential. We strongly encourage to use "General Protocol" first. If the "General Protocol" fails to give satisfactory result (e.g., less than 10%), try the "Advanced Protocol" on back page.
- For high efficiency and lower toxicity, transfect cells at high density. ~90% confluency is highly recommended.
- To lower cytotoxicity, transfect cells in presence of serum (10%) and antibiotics.

PART I. A General Protocol for Transfecting Caco-2 Cells:

Step I. Cell Seeding:

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 anti-biotics is freshly added to each well ~60 minutes before transfection.

Table 1. 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
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	5 - 6	2 x 0.5	15 - 18
T75 flask	6	9 - 12	2 x 0.75	27 - 36
250 ml flask	12	30 - 50	2 x 1.25	90 - 150

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

For Caco-2 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 1** for transfection in other culture formats. The optimal transfection conditions For Caco-2 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 DNA and GenJet™ reagent as 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 or vortex briefly 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 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 ~24 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

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Part II. Advanced Protocol for Transfecting Hard-To-Transfect Mammalian Cells

Important: The advanced protocol for hard-to-transfect cells is provided only if general protocol gives less than 10% efficiency.

Step I. Culturing of Cells Before Transfection:

Cells should be plated at least 24 hours prior to transfection so that the monolayer cell density reaches to the optimal 95~100% confluency at the day of transfection.

Table 2. A Guideline for Optimal Cell Number Per Well in Different Culture Formats

Culture Dishes	Surface Area (cm^2)	Optimal Cell Number
T75 Flask	75	9.6×10^6
100 mm Dish	58	7.3×10^6
60 mm Dish	21	2.7×10^6
35 mm Dish	9.6	1.0×10^6
6-well Plate	9.6	1.0×10^6
12-well Plate	3.5	0.44×10^6
24-well Plate	1.9	0.24×10^6
48-well Plate	1.0	0.11×10^6
96-well Plate	0.3	0.31×10^5

Table 3. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Transfection Complex Volume (ml)	Plasmid DNA (µg)	GenJet™ Reagent (µL)
96-well	0.02	0.2	0.8
48-well	0.04	0.5	2
24-well	0.1	1	4
12-well	0.12	1.2	4.8
6-well	0.2	2	8
35 mm dish	0.2	2	8
60 mm dish	0.5	5	20
10 cm dish	1.0	7	28
T75 flask	1.5	15	60
250 ml flask	2.5	50	200

Step II. Preparation of Cells in Suspension:

The following protocol is given for transfecting hard-to-transfect cells in 6-well plates, refer to **Table 2** for optimal cell number per well per culture vessels' surface area. The optimal ransfection conditions are given in the standard protocol described below. Detach the cells with trypsin/EDTA and stop the trypsinization with complete culture medium.

Note: Cells that are difficult to detach may be placed at 37 °C for 5-15 min to facilitate detachment.

- Take an aliquot of trypsinized cell suspension and count the cells to determine the cell density.
- Centrifuge the required ~1.0x106 cells per well for 6-well plate at 150xg at room temperature for 10 min.
- Use fine tip pipette to remove supernatant **completely** so that no residual medium covers the cell pellet.

Step III. Preparation and application of transfection complex:

For most of mammalianells, the optimal ratio of GenJet™ (µL):DNA (µg) is 4:1. 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 6-well plates, refer to **Table 3** for transfection in other culture formats.

- For each well of 6-well plate, dilute 2 µg of DNA into 100 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 of 6-well plate, dilute 8 µl of GenJet™ reagent into 100 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.

Note: Never use Opti-MEM to dilute DNA and GenJet™ reagent as 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 or vortex briefly to mix followed by incubation of 10~15 minutes at room temperature to allow GenJet™-DNA transfection complexes to form.

Note: Never keep the transfection complexes longer than 30 minutes

- **Gently** resuspend the cell pellet prepared from **Step II** immediately in the 200 µl transfection complex and incubate at 37 °C for 20 minutes.
- At the end of incubation, add 2.0 ml of pre-warmed fresh complete cell growth medium to cells and plate onto one well of a 6-well plate. Incubate at 37 °C with 5% CO2.
- Change culture medium **gently** 16~24 hours post transfection and check transgen expression.