Cat # SL100489-3T3 Store at 4 °C

GenJet™ In Vitro DNA Transfection Reagent for NIH 3T3 Cells (Ver. II)

----- Protocols for Transfecting NIH-3T3 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 DAN delivery. GenJet™ (Ver. II) for NIH 3T3 is pre-optimized and pre-conditioned for transfecting NIH 3T3 cells. We offer a general protocol and an advanced protocol for NIH 3T3 cells and its hard-to-transfect derivatives.

A General Protocol for Transfecting NIH-3T3 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 ~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. 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	1.2	2	2 x 0.1	6
35 mm dish	1.2	2	2 x 0.1	6
60 mm dish	3	5	2 x 0.25	15
10 cm dish	6	7 - 8	2 x 0.5	21 - 24
T75 flask	10	16 - 18	2 x 0.75	48 - 54
250 ml flask	20	30 - 50	2 x 1.25	90 - 150

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

For Nih-3t3 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 NIH-3T3 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 will 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 !)**
- 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 GenJet™-DNA complexes 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 ~5 hours post transfection.
- Check transfection efficiency 24 to 48 hours post transfection.

An Advanced Protocol for Transfecting Hard-to-transfect NIH-3T3 Derivatives:

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.

Step II. Preparation of Cells in Suspension

The following protocol is given for transfecting NIH-3T3 derivatives in 6-well plates. The optimal transfection conditions for hard-to-transfect NIH-3T3 derivatives 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 $1.2x10^6$ cells per well for 6-well plate at 150xg at room temperature for 10 min. Refer to **Table 3** for optimal cell numbers for other culture formats.

Cat # SL100489-N2A Store at 4 °C

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- 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

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 2** 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 (Ver. II) into 100 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 will 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 !)**
- Vortex-mix the solution immediately and spin down briefly to bring drops to bottom of the tube followed by incubation of 10~15 min at room temperature to allow transfection complexes to form.

  Note: Never keep the transfection complexes longer than 20 min
- Resuspend the cell pellet prepared from **Step II** immediately in the 200 µl transfection complex and incubate at 37 °C for 20 min.
- 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.
- Check transfection efficiency 24 to 48 hours post transfection.

Table 2. 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	1.0	4
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	10	40
250 ml flask	2.5	40	160

Table 3. 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	1.2×10^6
6-well Plate	9.6	1.2×10^6
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