SKU # 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 Transfection Reagent (Ver. II) is an upgraded version of the original GenJet™ In Vitro DNA Transfection Reagent. Through improved proprietary chemistry, the new formulation contains a higher density of DNA-condensing functional groups than the original GenJet™ reagent, resulting in 3- to 20-fold greater DNA delivery efficiency. GenJet™ (Ver. II) for NIH 3T3 cells has been pre-optimized and pre-conditioned specifically for NIH 3T3 cells. We provide both a general protocol and a reverse transfection protocol for the transfection of NIH 3T3 cells and their hard-to-transfect derivatives. We recommend using the reverse transfection protocol only if the general protocol fails to achieve satisfactory transfection efficiency.

A General Protocol for Transfecting NIH-3T3 Cells

Step I. Cell Seeding

Seed cells 18–24 hours prior to transfection so that they reach approximately 60% confluency at the time of transfection. For optimal transfection performance, replace the culture medium with fresh complete growth medium containing serum and antibiotics approximately 60 minutes before transfection.

Table 1. Recommended Transfection Conditions for Various Culture Vessel Formats

Culture Dish	Culture Medium Volume (mL)	Plasmid DNA (µg)	Serum-Free Diluent Volume (µL)	GenJet™ Reagent Ver. II (µL)
48 well plate	0.5	0.25	30	0.75
12 well plate	0.75	0.75	70	2.25
6-well plate	2.0	1.5	100	4.5
35 mm dish	2.0	1.5	100	4.5
60 mm dish	5	2.5	200	7.5
10 cm plate	10	6	500	18
T75 flask	10	6	500	18
15 cm plate	20	18	1000	54

Step II. Preparation of GenJet™–DNA Complexes and Transfection Procedure

The optimal GenJet™ (µL) to DNA (µg) ratio is 3:1. To promote the formation of optimally sized DNA complexes, we recommend using serum-free high-glucose DMEM for dilution of both DNA and GenJet™ reagent.

The following protocol is provided for transfection in 24-well plates. For other culture formats, refer to **Table 1**. The optimal transfection conditions for NIH-3T3 cells are described in the general protocol below.

- **Medium Preparation:** Aspirate existing medium from each well and replace with 1.0 mL of fresh complete medium containing serum and antibiotics 30–60 minutes prior to transfection.
- **DNA Dilution:** For each well, add 0.5 µg of plasmid DNA to 50 µL of serum- and antibiotic-free culture medium in a 1.5 mL microcentrifuge tube. Gently mix by pipetting up and down 3–4 times.
- **GenJet™ Addition:** Add 1.5 µL of GenJet™ reagent directly to the diluted DNA solution. Gently mix immediately by pipetting up and down 3–4 times.

  ⚠ ***Critical:*** *Do not use Opti-MEM as the diluent for either the DNA or GenJet™ reagent. Opti-MEM disrupts complex formation and significantly reduces transfection efficiency.*
- **Complex Formation:** Incubate the transfection mix at room temperature for 15–20 minutes to allow GenJet™–DNA complexes to form.

  ⚠ ***Critical:*** *Do not exceed 20 minutes of incubation. Prolonged incubation leads to aggregate formation and reduced transfection efficiency.*
- **Transfection:** Add the entire volume of GenJet™–DNA complex dropwise onto the cells in each well. Gently swirl the plate to ensure uniform distribution of the complex across the cell monolayer. Return the plate to the 37 ºC / 5% CO_2 incubator immediately.
- **Medium Replacement:** Approximately 18 hours post-transfection, aspirate the complex-containing medium and replace with fresh complete medium supplemented with serum and antibiotics.
- **Assessment of Transfection Efficiency:** Transfection efficiency should be evaluated 24–48 hours post-transfection

A Reverse Transfection Protocol for NIH-3T3 Cells

Step I. Preparation of Cells in Suspension

The following protocol is provided for reverse transfection of NIH-3T3 derivatives in 6-well plates. For other tissue culture formats, refer to **Table 2**.

- Culture NIH-3T3 cells in an appropriately sized tissue culture vessel (e.g., a 10-cm dish) until they reach approximately 100% confluency.
- Detach the cells using trypsin-EDTA and neutralize the enzyme by adding complete growth medium.
- Take a small aliquot of the cell suspension and determine the cell density using an appropriate cell-counting method.
- Pellet 1.0×10^6 cells per well (6-well plate format) by centrifugation at 150 × g for 10 minutes at room temperature. Refer to **Table 3** for the recommended cell numbers for other culture vessel formats.
- Carefully remove the supernatant using a fine-tip pipette, ensuring that no residual medium remains covering the cell pellet.

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Step II. Preparation and Application of Transfection Complexes

The optimal GenJet™ (µL) to DNA (µg) ratio is 4:1. To promote the formation of optimally sized transfection complexes, we recommend using serum-free high-glucose DMEM for dilution of both DNA and GenJet™ reagent.

The following protocol is provided for transfection in 6-well plates. For other culture formats, refer to **Table 2.**

- For each well of a 6-well plate, dilute 2 µg of plasmid DNA in 200 µL of serum-free high-glucose DMEM. Gently vortex and briefly centrifuge to collect the solution at the bottom of the tube.
- Add 8 µL of GenJet™ Reagent (Ver. II) to the above diluted DNA solution. Gently mix by pipetting up and down several times.
  Note: *Do not use Opti-MEM to dilute GenJet™ reagent or DNA, as it may interfere with transfection complex formation.*
- Incubate for 10–15 minutes at room temperature to allow transfection complexes to form.
  Note: *Do not incubate the transfection complexes for longer than 20 minutes.*
- Immediately resuspend the cell pellet prepared in **Step I** in the 200 µL transfection complex followed by gently pipetting up and down to mix and incubate at 37°C for 20 minutes.
- At the end of the incubation, add 2.0 mL of pre-warmed complete growth medium and transfer the cells to one well of a 6-well plate.
- Assess transfection efficiency 24–48 hours post-transfection.

Table 2. Recommended Transfection Conditions for Various Culture Vessel Formats

Culture Dish	Culture Medium Volume (mL)	Plasmid DNA (µg)	Serum-Free Diluent Volume (µL)	GenJet™ Reagent Ver. II (µL)
48 well plate	0.5	0.25	30	1.0
24 well plate	1.0	1.0	100	4.0
12 well plate	1.5	1.5	150	6
6-well plate	2.0	2	200	8.0
35 mm dish	2.0	2	200	8.0
60 mm dish	5	3	300	12
10 cm plate	10	5	500	20
T75 flask	10	5	500	20
15 cm plate	20	15	1500	60

Table 3. Recommended Cell Numbers per Well for Different Culture Vessel Formats

Culture Dish	Surface Area (cm^2)	Optimal Cell Number
48 well plate	1.0	0.11×10^6
24 well plate	1.9	0.24×10^6
12 well plate	3.5	0.44×10^6
6-well plate	9.6	1.0×10^6
35 mm dish	9.6	1.0×10^6
60 mm dish	21	2.7×10^6
10 cm plate	58	7.3×10^6
T75 flask	75	7.3×10^6
15 cm plate	145	15×10^6

Storage: GenJet™ Reagent Ver. II is stable for 24 months at +4 °C. This item shipped at ambient temperature