Cat # SL100489-SAOS Store at 4 °C

GenJet™ In Vitro DNA Transfection Kit for SaoS-2 Cells (Ver. II)

----- An Advanced Protocol for Transfecting SaoS-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 3~4 times more efficient in DAN delivery. In combination of a proprietary transfection toxicity removal cocktail, GenJet™ In Vitro Transfection Kit (Ver. II) for SaoS-2 cells is pre-optimized and pre-conditioned for maximally transfecting SaoS-2 cells.

Contents Per Kit:

1. 1x1.0 ml of GenJet™ DNA Transfection Reagent (Ver. II)
2. 1x1.0 ml (x50) of HapiCell™ Transfection Toxicity Removal Cocktail

Procedures for Transfecting SaoS-2 Cells:

Step I. Preparation Working Solution of HapiCell™ Transfection Toxicity Removal Cocktail

HapiCell™ Transfection Toxicity Removal Cocktail is provided as 50x stock solution. Before performing transfection, prepare working solution by mixing the provided 1.0 ml stock solution with 49.0 ml of PBS (without calcium and magnesium) in a sterile bottle. The working solution is stable for years under storage at 4 °C and should be left at room temperature two hours before use.

Step II. Cell Seeding

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 1. A Guideline for Seeding SaoS-2 Cells Prior to Transfection 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

Step III. Preparation of Cells in Suspension

The following protocol is given for transfecting SaoS-2 cells in 6-well plates, refer to **Table 1** for optimal cell number per well per culture vessels' surface area.

The optimal transfection conditions for mammalian cells 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 1.2×10^6 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.

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	8	32
T75 flask	1.5	36	144
250 ml flask	2.5	100	400

Step IV. 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.
- Add the diluted GenJet™ Plus Reagent **immediately** to the diluted DNA solution all at once.
- Vortex-mix the solution immediately and spin down briefly to bring drops to bottom of the tube followed by incubation of 10~15 minutes at room temperature to allow transfection complexes to form.

  Important: Never keep the transfection complexes longer than 20 minutes
- **Gently** resuspend the cell pellet prepared from Step III immediately in the 200 µl transfection complex and incubate at

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37 °C for 20 minutes.

- At the end of incubation, add 2.0 ml of pre-warmed fresh complete cell growth medium (with 10% FBS and antibiotics) to cells and plate onto one well of a 6-well plate. Incubate at 37 °C / 5% CO_2.
- **Gently** remove DNA/GenJet™ complex-containing medium short after SaoS-2 cells attaching the cell culture dish (around 8 hours after plating SaoS-2 cells), then briefly rinse SaoS-2 cells with PBS followed by 2 minutes incubation with Working Solution of HapiCell™ Transfection Toxicity Removal Cocktail at RT with gentle agitation.
- Aspirate the toxicity removal working solution after 2 minutes incubation at RT and rinse once more briefly with PBS followed by addition of complete serum/antibiotics containing medium.
- 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