

# GenJet™ In Vitro DNA Transfection Kit for Saos-2 Cell

----- A Standard Protocol for Transfecting Saos-2 Cell

- 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 DAN delivery. In combination of a proprietary transfection toxicity removal cocktail, GenJet™ In Vitro Transfection Kit (Ver. II) for Saos-2 is pre-optimized and pre-conditioned for maximally transfecting Saos-2 cells without visible cell death.

## Contents Per Kit:

- 1 x 1.0 ml of GenJet™ DNA Transfection Reagent for Saos-2 Cell.
- 1 x 8.0 ml (5x ) of GenJet™ Transfection Buffer.

## Procedures for Transfecting Saos-2 Cell:

### Step I. Preparation of Working Solution of GenJet™ Transfection Buffer

GenJet™ Transfection Buffer (5x ) is provided as 5 times concentrated stock solution. To make working solution, dilute one part of the stock solution with 4 parts of ddH<sub>2</sub>O. The 1x GenJet™ Transfection Buffer is table at 4 °C-RT for 24 months.

**Note:** Always keep GenJet™ Transfection Buffer (5x ) at RT. If refrigerated, white precipitates may appear. It won't affect the transfection efficiency. After dilution with 4 parts of ddH<sub>2</sub>O to make GenJet™ Transfection Buffer (1x ) working solution, the white precipitates will disappear.

### Step 2. 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 ~70% 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 <sup>2</sup> )	Number of Cells to Seed
T75 Flask	75	3.0 - 6.0 × 10 <sup>6</sup>
100 mm Dish	58	2.2 - 4.4 × 10 <sup>6</sup>
60 mm Dish	21	0.9 - 1.8 × 10 <sup>6</sup>
35 mm Dish	9.6	3.5 - 7.0 × 10 <sup>5</sup>
6-well Plate	9.6	4.0 - 8.0 × 10 <sup>5</sup>
12-well Plate	3.5	1.5 - 3.0 × 10 <sup>5</sup>
24-well Plate	1.9	0.8 - 1.6 × 10 <sup>5</sup>
48-well Plate	1.0	4.0 - 8.0 × 10 <sup>4</sup>
96-well Plate	0.3	1.2 - 2.4 × 10 <sup>4</sup>

### Step 3. Preparation of GenJet™-DNA Complex and Transfection Procedures

For Saos-2 cell, the optimal ratio of GenJet™ (µL):DNA (µg) is 2.25:1. To ensure the optimal size of complex particles, we recommend using 1x GenJet™ Transfection Buffer to dilute DNA and GenJet™ Reagent.

The following protocol is given for transfection in 6-well plate, refer to [Table 2](#) for transfection in other culture formats. The optimal transfection conditions for Saos-2 are given in the standard protocol described below.

Table 2. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Culture Medium (ml)	Plasmid DNA (µg)	Transfection Buffer (1x ) (mL)	GenJet™ Reagent (µL)
96-well	0.1	0.1	0.010	0.225
48-well	0.25	0.25	0.025	0.5625
24-well	0.5	0.5	0.050	1.125
6-well	1	2.0	0.20	4.50
35 mm dish	1	2.0	0.20	4.50
60 mm dish	3	4.0	0.40	9.0
10 cm dish	6	10	1.0	22.5
T75 flask	6	11	1.1	24.75

- For each well, dilute 2.0 µg of DNA into 200 µl of 1xGenJet™ Transfection Buffer prepared from [Step 1](#). Pipetting up and down to mix.
- Add 4.5 µl of GenJet™ reagent (Ver. II) into the diluted plasmid DNA. Vortex briefly to mix.
- Incubate for ~10 min at room temperature to allow GenJet™-DNA complexes to form.
- Note:** Never keep GenJet™-DNA complexes longer than 20 min.
- Add the 200 µl GenJet™/ DNA complex drop-wise onto the cell culture and homogenize the mixture by gently swirling the plate.
- Remove DNA/GenJet™ complex-containing medium after overnight incubation followed by addition of complete serum/antibiotics containing medium.
- Check transfection efficiency 24 to 48 hours post transfection.

**Storage:** GenJet™ In Vitro Transfection Kit is stable for up to 12 months at 4 °C. This item shipped at ambient temperature