Cat # SL100489-SHEP Store at 4 °C

GenJet™ In Vitro DNA Transfection Reagent for SHEP Cell (Ver. II)

----- A Protocol for Transfecting SHEP Cells

- ☐ 100 µl
- ☐ 500 µl
- ☐ 1000 µl

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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 SHEP cell is pre-optimized and pre-conditioned for transfecting SHEP cells.

Procedures for Transfecting SHEP Cells:

Step I. 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 ~85% 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^2)	Number of Cells to Seed
T75 Flask	75	$3.0 – 6.0 \times 10^6$
100 mm Dish	58	$2.2 – 4.4 \times 10^6$
60 mm Dish	21	$0.9 – 1.8 \times 10^6$
35 mm Dish	9.6	$3.5 – 7.0 \times 10^5$
6-well Plate	9.6	$4.0 – 8.0 \times 10^5$
12-well Plate	3.5	$1.5 – 3.0 \times 10^5$
24-well Plate	1.9	$0.8 – 1.6 \times 10^5$
48-well Plate	1.0	$4.0 – 8.0 \times 10^4$
96-well Plate	0.3	$1.2 – 2.4 \times 10^4$

Table 2. 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	0.15	2 x 0.01	0.45
48-well	0.3	0.3	2 x 0.02	0.6
24-well	0.5	0.75	2 x 0.05	2.25
6-well	1.0	1.5	2 x 0.1	4.5
35 mm dish	1.0	1.5	2 x 0.1	4.5
60 mm dish	3	4	2 x 0.25	12
10 cm dish	5	7	2 x 0.5	21
T75 flask	6	11	2 x 0.75	33
250 ml flask	11	30	2 x 1.25	90

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

For SHEP 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 2** for transfection in other culture formats. The optimal transfection conditions for SHEP 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 0.75 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 2.25 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 !)**
- 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 16~24 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