Cat # SL100489 Store at 4 °C

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

----- A Protocol for generation of Lentivirus from 293T 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 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) was shown to generate lentivirus with extremelt high titers from 293T cells.

Important Transfection Guidelines for New Version:

- Do NOT follow transfection procedures for GenJet old version. Read protocol for new version carefully before transfection
- For high efficiency, transfect cells at high density. 90~95% confluency is highly recommended
- To lower cytotoxicity, transfect cells in presence of serum (10%) and antibiotics
- Change medium with serum (10%) and antibiotics 5 hours post transfection is optional

Procedures for Transfecting 293T Cells:

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 ~95% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30~60 minutes before transfection.

Note: High serum levels (>5%) with antibiotics usually do not have inhibitory effect on transfection efficiency. For some specific 293 cells, maximal transfection efficiencies are observed in the presence of serum and antibiotics. We recommend using complete serum/antibiotics-containing medium initially.

Table 1. A Guideline for Seeding Adherent Cells Prior to Transfection in Different Culture Formats.

Culture Dishes	Surface Area (cm2)	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	$1.2 – 2.4 \times 10^4$

Preparation of GenJet™-DNA Complex and Transfection Procedures

The following protocol is given for transfection in 10 cm dish. For other culture formats, scale up or down per culture dish's surface. The optimal transfection conditions are given in the standard protocol described below.

- Cell confluency should be ~90 % at the day of transfection
- For each 10 cm dish, add 5.0 ml of complete medium with serum and antibiotics freshly 30~60 minutes before transfection.
- For each dish, dilute total 15 µg of DNA (three DNAs with optimal ratio) into 500 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly to bring drops to bottom of the tube .
- For each dish, dilute 45 µl of GenJet™ reagent (Ver. II) into 500 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 because 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 15~20 minutes at room temperature to allow GenJet™-DNA complexes to form.
  Note: Never keep the DNA/GenJet™ complex longer than 20 minutes
- Add the 1000 µl GenJet™/ DNA complex drop-wise onto the medium in each dish 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 and virus titer 24 to 48 hours post transfection. 48 hours gives better titers.

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