Store at 4 °C

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

----- A Protocol for generation of rAAV 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 rAAV 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. 80~90% 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 80~90% 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 x 10^6
100 mm Dish	58	2.2 – 4.4 x 10^6
60 mm Dish	21	0.9 – 1.8 x 10^6
35 mm Dish	9.6	3.5 – 7.0 x 10^5
6-well Plate	9.6	4.0 – 8.0 x 10^5
12-well Plate	3.5	1.5 – 3.0 x 10^5
24-well Plate	1.9	0.8 – 1.6 x 10^5
48-well Plate	1.0	4.0 – 8.0 x 10^4
96-well Plate	0.3	1.2 – 2.4 x 10^4

Preparation of GenJet™-DNA Complex and Transfection Procedures

The following protocol is given for transfection in 150 mm 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 80~90 % at the day of transfection
- For each 10 cm dish, add 15 ml of complete medium with serum and antibiotics freshly 30~60 minutes before transfection.
- For each dish, dilute 10 µg of rAAV cis plsamid, 10 µg capsid DNA and 16 µg helper DNA (total 36 µg DNA) in 750 µl serum-free DMEM with high glucose. Vortex briefly to mix.
- For each dish, dilute 100 µl of GenJet™ reagent (Ver. II) into 750 µl of serum-free DMEM with High Glucose. Vortex gently to mix.
  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 !)**
- Immediately pipette up and down 3~4 times or vortex briefly to mix followed by incubation for ~10 minutes at room temperature to allow GenJet™-DNA transf-ection complexes to form.
  Note: Never keep the DNA/GenJet™ complex longer than 20 minutes
- Add the 1500 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