Cat # SL100489-PC12 Store at 4 °C

GenJet™ In Vitro DNA Transfection Reagent for PC-12 Cell (Ver. II)

----- A Protocol for Transfecting PC-12 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 DNA delivery. GenJet™ (Ver. II) for PC-12 cell was pre-optimized and conditioned for transfecting PC-12 cells.

Procedures for Transfecting PC-12 Cells:

Step I. Cell Seeding (see Table 1):

Undifferentiated adherent PC-12 cells should be cultured in DMEM supplemented with 10% horse serum (HS) and 5% fetal bovine serum (FBS). PC-12 cellss should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal ~80% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well ~60 minutes before transfection. Transfection of differentiated PC-12 cells required prior stimulation of adherent PC-12 cells with 50 ng/ml NGF.

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	0.2	2 x 0.01	0.6
48-well	0.3	0.5	2 x 0.02	1.5
24-well	0.5	1.0	2 x 0.05	3
35 mm dish	1.0	2	2 x 0.1	6
60 mm dish	3	5	2 x 0.25	15
10 cm dish	6	7 - 8	2 x 0.5	21 - 24
T75 flask	6	10 - 15	2 x 0.75	30 - 45
250 ml flask	12	30 - 50	2 x 1.25	90 - 150

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

For PC-12 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 6-well plates, refer to **Table 2** for transfection in other culture formats. The optimal transfection conditions For PC-12 cells are given in the standard protocol described below.

- For each well, add 1.0 ml of complete medium with serum and antibiotics freshly ~60 minutes before transfection.
- For each well, 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, dilute 6 µl of GenJet™ reagent (Ver. II) into 100 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.
  Note: Never use Opti-MEM to dilute GenJet™ reagent and DNA, 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 minutes at room temperature to allow GenJet™-DNA complexes to form.
  Note: Never keep the DNA/GenJet™ complex longer than 20 minutes
- Add the 200 µl GenJet™/ DNA complex drop-wise onto the medium in each well and homogenize the mixture by gently swirling the plate.
- Change medium 16~24 hours after transfection.
- Check transgene expression 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

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