Cat # SL100489-N2A   Store at 4 °C

GenJet™ In Vitro DNA Transfection Reagent for Neuro-2A Cells (Ver. II)

----- A Protocol for Transfections of Neuro-2A Cells

☐ 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 3~4 times more efficient in DAN delivery. GenJet™ (Ver. II) for Neuro-2A is pre-optimized and pre-conditioned for transfecting Neuro-2A cells.

Procedures for Transfecting Neuro-2A Cells:

Step A. 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 ~90% confluency at the time of transfection. Complete culture medium with serum and anti-biotics 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	0.2	2 x 0.01	0.6
48-well	0.3	0.5	2 x 0.02	1
24-well	0.5	1.0	2 x 0.05	3
6-well	1.2	2	2 x 0.1	6
35 mm dish	1.5	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	10	18 - 36	2 x 0.75	54 - 108
250 ml flask	20	50 - 100	2 x 1.25	150 - 300

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

For Neuro-2A 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 Neuro-2A 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 1 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 3 µl of GenJet™ reagent (Ver. II) into 50 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.
- 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 tempera-ture 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 ~5 hours post transfection.
- Check transfection efficiency 24 to 48 hours post transfection.

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

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