Cat # SL100489-N2A Store at 4 °C

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

----- A Protocol for Transfecting 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. We offer a general protocol and an advanced protocol for Neuro-2A cells and its hard-to-transfect derivatives.

A General Protocol for Transfecting Neuro-2A Cells:

Step I. Cell Seeding

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal ~70% 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. 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	1.2	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 - 25	2 x 0.75	54 - 75
250 ml flask	20	50 - 70	2 x 1.25	150 - 210

Step II. 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 1** 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.
  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 GenJet™-DNA complexes longer than 30 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.