SKU # SL100489-N2A Store at 4 °C

# GenJet™ In Vitro DNA Transfection Reagent for Neuro2A Cells (Ver. II)

----- A Protocol for Transfecting Neuro2A 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 Transfection Reagent (Ver. II) is an upgraded version of the original GenJet™ In Vitro DNA Transfection Reagent. Through improved proprietary chemistry, the new formulation contains a higher density of DNA-condensing functional groups than the original GenJet™ reagent, resulting in 3- to 20-fold greater DNA delivery efficiency. GenJet™ (Ver. II) for Neuro2A cells has been pre-optimized and pre-conditioned specifically for Neuro2A cells. We provide a general protocol for transfecting Neuro2A cells using a ~5.0 kb GFP expression plasmid as a reporter. Because transfection efficiency may vary with plasmid size and construct design, we recommend optimizing the transfection conditions for individual plasmids to achieve maximal transfection efficiency and transgene expression.

**A General Protocol for Transfecting the Neuro2A Cells**

**Step I. Cell Seeding**

Seed cells 18–24 hours prior to transfection so that they reach approximately 60% confluency at the time of transfection. For optimal transfection performance, replace the culture medium with fresh complete growth medium containing serum and antibiotics approximately 60 minutes before transfection.

**Table 1. Recommended Transfection Conditions for Various Culture Vessel Formats**

| Culture Dish | Culture Medium Volume (mL) | Plasmid DNA (µg) | Serum-Free Diluent Volume (µL) | GenJet™ Reagent Ver. II (µL) |
|---|---|---|---|---|
| 48 well plate | 0.5 | 0.25 | 25 | 0.75 |
| 12 well plate | 0.75 | 0.75 | 75 | 2.25 |
| 6-well plate | 2.0 | 1.0 | 100 | 3.0 |
| 35 mm dish | 2.0 | 1.0 | 100 | 3.0 |
| 60 mm dish | 5 | 3 | 300 | 9 |
| 10 cm plate | 10 | 6 | 600 | 18 |
| T75 flask | 10 | 6 | 600 | 18 |
| 15 cm plate | 20 | 15 | 1000 | 45 |

**Step II. Preparation of GenJet™–DNA Complexes and Transfection Procedure**

The optimal GenJet™ (µL) to DNA (µg) ratio is 3:1. To promote the formation of optimally sized DNA complexes, we recommend using serum-free high-glucose DMEM for dilution of both DNA and GenJet™ reagent.

The following protocol is provided for transfection in 24-well plates. For other culture formats, refer to **Table 1**. The optimal transfection conditions for Neuro2A cells are described in the general protocol below.

- **Medium Preparation:** Aspirate existing medium from each well and replace with 1.0 mL of fresh complete medium containing serum and antibiotics 30–60 minutes prior to transfection.
- **DNA Dilution:** For each well, add 0.5 µg of plasmid DNA to 50 µL of serum- and antibiotic-free culture medium in a 1.5 mL microcentrifuge tube. Gently mix immediately by pipetting up and down 3–4 times.
- **GenJet™ Addition:** Add 1.5 µL of GenJet™ reagent directly to the diluted DNA solution. Gently mix immediately by pipetting up and down 3–4 times.

  ⚠ ***Critical:*** *Do not use Opti-MEM as the diluent for either the DNA or GenJet™ reagent. Opti-MEM disrupts complex formation and significantly reduces transfection efficiency.*
- **Complex Formation:** Incubate the transfection mix at room temperature for 15–20 minutes to allow GenJet™–DNA complexes to form.

  ⚠ ***Critical:*** *Do not exceed 20 minutes of incubation. Prolonged incubation leads to aggregate formation and reduced transfection efficiency.*
- **Transfection:** Add the entire volume of GenJet™–DNA complex dropwise onto the cells in each well. Gently swirl the plate to ensure uniform distribution of the complex across the cell monolayer. Return the plate to the 37 ºC / 5% $CO_2$ incubator immediately.
- **Medium Replacement:** Approximately 18 hours post-transfection, aspirate the complex-containing medium and replace with fresh complete medium supplemented with serum and antibiotics.
- **Assessment of Transfection Efficiency:** Transfection efficiency should be evaluated 24–48 hours post-transfection

**Storage:** GenJet™ Reagent Ver. II is stable for 24 months at +4 °C. This item shipped at ambient temperature