Cat # SL100489-C2C12 Store at 4 °C

# GenJet™ In Vitro DNA Transfection Reagent for C2C12 Cells (Ver. II)

----- A Protocol for Transfecting C2C12 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 3~20 times more efficient in DNA delivery. GenJet™ (Ver. II) for C2C12 cells was pre-optimized and conditioned for transfecting C2C12 cells.

## Procedures for Transfecting C2C12 Cells:

### Step I. Culturing of Cells Before Transfection

Cells should be plated at least 24 hours prior to transfection so that the monolayer cell density reaches to the optimal 95~100% confluency at the day of transfection.

**Table 1. A Guideline for Optimal Cell Number Per Well in Different Culture Formats**

| Culture Dishes | Surface Area ($cm^2$) | Optimal Cell Number |
|---|---|---|
| T75 Flask | 75 | $9.6 \times 10^6$ |
| 100 mm Dish | 58 | $7.3 \times 10^6$ |
| 60 mm Dish | 21 | $2.7 \times 10^6$ |
| 35 mm Dish | 9.6 | $1.2 \times 10^6$ |
| 6-well Plate | 9.6 | $1.2 \times 10^6$ |
| 12-well Plate | 3.5 | $0.44 \times 10^6$ |
| 24-well Plate | 1.9 | $0.24 \times 10^6$ |
| 48-well Plate | 1.0 | $0.11 \times 10^6$ |
| 96-well Plate | 0.3 | $0.31 \times 10^5$ |

**Table 2. Recommended Amounts for Different Culture Vessel Formats**

| Culture Dish | Transfection Complex Volume (ml) | Plasmid DNA (µg) | GenJet™ Reagent (µL) |
|---|---|---|---|
| 96-well | 0.02 | 0.2 | 0.8 |
| 48-well | 0.04 | 0.5 | 2 |
| 24-well | 0.1 | 1.0 | 4 |
| 6-well | 0.2 | 2 | 8 |
| 35 mm dish | 0.2 | 2 | 8 |
| 60 mm dish | 0.5 | 5 | 20 |
| 10 cm dish | 1.0 | 8 | 32 |
| T75 flask | 1.5 | 36 | 144 |
| 250 ml flask | 2.5 | 100 | 400 |

### Step II. Preparation of Cells in Suspension

The following protocol is given for transfecting C2C12 in 6-well plates, refer to **Table 1** for optimal cell number per well per culture vessels' surface area. The optimal transfection conditions for C2C12 cells are given in the standard protocol described below.

- Detach the C2C12 cells with trypsin/EDTA and stop the trypsinization with complete culture medium.
  **Note:** Cells that are difficult to detach may be placed at 37 °C for 5-15 min to facilitate detachment
- Take an aliquot of trypsinized cell suspension and count the cells to determine the cell density.
- Centrifuge the required $1.2 \times 10^6$ cells per well for 6-well plate at 160xg at room temperature for 10 min.
- Use fine tip pipette to remove supernatant **completely** so that no residual medium covers the cell pellet.

### Step III. Preparation and application of Transfection Complex

**For C2C12 cells, the optimal ratio of GenJet™ (µL):DNA (µg) is 4:1. 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.

- For each well of 6-well plate, 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 of 6-well plate, dilute 8 µl of GenJet™ reagent (Ver. II) into 100 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 !)**
- Immediately pipette up and down 3~4 times or vortex briefly to mix followed by incubation for ~15 minutes at room temperature to allow GenJet™-DNA transfection complexes to form.
  **Note:** Never keep the transfection complexes longer than 20 minutes
- Resuspend the cell pellet prepared from **Step II** immediately in the 200 µl transfection complex and incubate at 37 °C for 20 minutes.
- At the end of incubation, add 2.0 ml of pre-warmed fresh complete cell growth medium to cells and plate onto one well of a 6-well plate.
- Check transfection efficiency 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 RT