Cat # SL100489-MEF Store at 4 °C

GenJet™ In Vitro DNA Transfection Reagent for MEFs (Ver. II)

----- A Protocol for Transfecting MEFs

☐ 100 µl
☐ 500 µl
☐ 1000 µl

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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 MEFs was pre-optimized and conditioned for transfecting mouse embryonic fibroblasts (MEFs).

Procedures for Transfecting MEFs Cells:

Step I. Culturing of Cells Before Transfection

We recommend growing MEFs in a 10 cm plate. The 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	9.4×10^6
100 mm Dish	58	7.3×10^6
60 mm Dish	21	2.7×10^6
35 mm Dish	9.6	1.2×10^6
6-well Plate	9.6	1.2×10^6
12-well Plate	3.5	0.42×10^6
24-well Plate	1.9	0.23×10^6
48-well Plate	1.0	0.13×10^6
96-well Plate	0.3	0.42×10^5

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	0.6
48-well	0.04	0.5	1.5
24-well	0.1	1.0	3
6-well	0.2	2	6
35 mm dish	0.2	2	6
60 mm dish	0.5	5	15
10 cm dish	1.0	7	21
T75 flask	1.5	10	30
250 ml flask	2.5	40	120

Step II. Preparation of Cells in Suspension

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

- Detach the MEFs cells with trypsin/EDTA and stop the trypsinization with complete culture medium containing 10% FBS.
  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 1.2×10^6 cells per well for 6-well plate at 150xg 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 MEFs 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.

- For each well of 6-well plate, dilute 2 µg of DNA into 100 µL of serum-free DMEM with High Glucose. Vortex gently to mix.
- For each well of 6-well plate, dilute 6 µL of GenJet™ reagent (Ver. II) into 100 µL of serum-free DMEM with High Glucose. Vortex gently to mix.
  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 followed by vortexing briefly to mix.
  Note: Do not mix the two solutions in the reverse order.
- Keep the transfection mix at RT for 10~15 min to allow 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 min.
- 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.
- Change medium next day.
- Check transfection efficiency 24 to 48 hours post transfection.

Storage: GenJet ™ Transfection Reagent is stable for up to 24 months at 4 °C.