Cat # SL100489-MCF10A Store at 4 °C

GenJet™ In Vitro DNA Transfection Reagent for MCF10A Cell (Ver. II)

----- Protocol for Transfecting MCF10A Cell

☐ 100 µl
☐ 500 µl
☐ 1000 µl

SignaGen® Laboratories
9601 Medical Center Drive
A/R Building, Suite 341
Rockville MD 20850
FAX. 301-560-4919
TEL. 301-330-5966
Toll Free. 1-(866)-918-6812
Email: info@signagen.com
Web: www.signagen.com

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 DAN delivery. GenJet™ (Ver. II) Reagent for MCF10A cell is pre-optimized and pre-conditioned for transfecting MCF10A cell.

A General Protocol for Transfecting MCF10A Cell:

The following protocol is given for growing the MCF10A cells in a 10 cm plate and transfecting the MCF10A cells in 6-well plates. For other cell culture formats, please scale up and down per the surface of tissue culture dish.

Step I. Cell Seeding

MCF10A cells should be plated in a 10 cm plate 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal 95~100% confluency at the time of transfection.

Step II. Preparation of Cells in Suspension

The following protocol is given for transfecting MCF10A derivatives in 6-well plates.

- Detach the MCF10A 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 optimal 1.2x10^6 cells per well for 6-well plate at 150xg at RT for 10 min.
  Refer to **Table 1** for optimal cell numbers for other culture formats.
- 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

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 to mix.
- For each well of 6-well plate, dilute 8 µL of GenJet™ reagent (Ver. II) reagent 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 may 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 gently to mix followed by incubation of the transfection mix for 5~10 min at RT to allow transfection complexes to form.
  Note: Never keep the transfection complexe longer than 15 min.
- 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 and check transfection efficiency 24 to 48 hours post 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 x 10^6
100 mm Dish	58	7.3 x 10^6
60 mm Dish	21	2.7 x 10^6
35 mm Dish	9.6	1.2 x 10^6
6-well Plate	9.6	1.2 x 10^6
12-well Plate	3.5	0.44 x 10^6
24-well Plate	1.9	0.24 x 10^6
48-well Plate	1.0	0.11 x 10^6
96-well Plate	0.3	0.31 x 10^5

Table 2. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Transfection Complex (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

Storage: GenJet™ Transfection Reagent Ver. II is stable for up to 12 months at 4 °C. This item shipped at ambient temperature