

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

## ----- Protocol for Transfecting MCF10A Cell

- 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 upgraded version of GenJet™ In Vitro DNA Transfection 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  $1.2 \times 10^6$  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 complex 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 <sup>2</sup> )	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 (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