

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

----- A Protocol for Transfecting MDCK 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 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 DNA delivery. GenJet™ (Ver. II) for MDCK cells was pre-optimized and conditioned for transfecting MDCK cells.

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

- Detach the MDCK 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 1.2x10⁶ 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 MDCK 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. Immediately pipette up and down to mix.
- 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. Immediately pipette up and down 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. **(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.