

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

----- A Protocol for Transfecting
COS-1 and COS-7 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 DAN delivery. GenJet™ (Ver. II) for COS cells was pre-optimized and conditioned for transfecting COS-1 and COS-7 cells.

Procedures for Transfecting COS Cells:

Step I. Cell Seeding (see Table 1):

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal ~80% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well ~60 minutes before transfection.

Table 1. A Guideline for Seeding Adherent Cells Prior to Transfection in Different Culture Formats

Culture Dishes	Surface Area (cm ²)	Number of Cells to Seed
T75 Flask	75	3.0 – 6.0 × 10 ⁶
100 mm Dish	58	2.2 – 4.4 × 10 ⁶
60 mm Dish	21	0.9 – 1.8 × 10 ⁶
35 mm Dish	9.6	3.5 – 7.0 × 10 ⁵
6-well Plate	9.6	4.0 – 8.0 × 10 ⁵
12-well Plate	3.5	1.5 – 3.0 × 10 ⁵
24-well Plate	1.9	0.8 – 1.6 × 10 ⁵
48-well Plate	1.0	4.0 – 8.0 × 10 ⁴
96-well Plate	0.3	1.2 – 2.4 × 10 ⁴

Table 2. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Transfection Volume (ml)	Plasmid DNA (µg)	Diluent Volume (mL)	GenJet Reagent (µL)
48 well plate	0.3	0.25	2 × 0.015	0.75
12 well plate	0.75	0.75	2 × 0.038	2.25
6-well plate	1.0	1	2 × 0.05	3.0
35 mm dish	1.0	1	2 × 0.05	3.0
60 mm dish	2.8	2.5	2 × 0.10	7.5
10 cm dish	5.0	3 - 4	2 × 0.25	9 - 12
T75 flask	8.0	9 - 18	2 × 0.40	27 - 54
250 ml flask	18	25 - 50	2 × 0.8	75 - 150

Step II. Preparation of GenJet™-DNA Complex and Transfection Procedures

For COS 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 24-well plates, refer to **Table 2** for transfection in other culture formats. The optimal transfection conditions For COS cells are given in the standard protocol described below.

- For each well, add 0.5 ml of complete medium with serum and antibiotics freshly ~60 minutes before transfection.
- For each well, dilute 0.5 µg of DNA into 25 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, dilute 1.5 µl of GenJet™ reagent (Ver. II) into 25 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.

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 !)**
- Vortex-mix the solution immediately and spin down briefly to bring drops to bottom of the tube followed by incubation of 15 minutes at room temperature to allow GenJet™-DNA complexes to form.

Note: Never keep the DNA/GenJet™ complex longer than 20 minutes

- Add the 50 µl GenJet™/ DNA complex drop-wise onto the medium in each well and homogenize the mixture by gently swirling the plate.
- Change medium 24 hours after transfection.
- Check transgene expression 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 ambient temperature