

GenJet™ In Vitro DNA Transfection Reagent for HuH-7 Cells (Ver. II)

----- A General Protocol for Transfecting HuH-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 3-4 times more efficient in DNA delivery. GenJet™ (Ver. II) for transfecting HuH-7 cells was formulated for transfection of HuH-7 cells.

Procedures for Transfecting HuH-7 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 ~90% confluency at the time of transfection. Complete culture medium with serum and anti-biotics is freshly added to each well ~60 minutes before transfection.

Note: 90% cell confluency at the day of transfection is essential for lower toxicity.

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 x 10 ⁶
100 mm Dish	58	2.2 - 4.4 x 10 ⁶
60 mm Dish	21	0.9 - 1.8 x 10 ⁶
35 mm Dish	9.6	3.5 - 7.0 x 10 ⁵
6-well Plate	9.6	4.0 - 8.0 x 10 ⁵
12-well Plate	3.5	1.5 - 3.0 x 10 ⁵
24-well Plate	1.9	0.8 - 1.6 x 10 ⁵
48-well Plate	1.0	4.0 - 8.0 x 10 ⁴
96-well Plate	0.3	1.2 - 2.4 x 10 ⁴

Table 2. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Culture Medium (ml)	Plasmid DNA (µg)	Diluent Volume (mL)	GenJet™ Reagent (µL)
96-well	0.2	0.2	2 x 0.01	0.6
48-well	0.3	0.5	2 x 0.02	1
24-well	0.5	1.0	2 x 0.05	3
6-well	1.0	2	2 x 0.1	6
35 mm dish	1.0	2	2 x 0.1	6
60 mm dish	3	5	2 x 0.25	15
10 cm dish	6	7 - 8	2 x 0.5	21 - 24
T75 flask	7	10 - 12	2 x 0.75	30 - 36
250 ml flask	12	30 - 50	2 x 1.25	90 - 150

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

For HuH-7 cells, the optimal ratio of GenJet™ (µL):DNA (µg) is 3:1. We recommend the GenJet™ (µL):DNA (µg) ratio of 3:1 as a starting point which usually gives satisfactory transfection efficiency with invisible cytotoxicity. 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 HuH-7 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 1 µg of DNA into 50 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 3 µl of GenJet™ reagent (Ver. II) into 50 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 !**)
- Immediately pipette up and down 3-4 times to mix followed by incubation for ~15 minutes at room temperature to allow GenJet™-DNA complexes to form.
- Note:** Never keep the DNA/GenJet™ complex longer than 30 minutes
- Add the 100 µl GenJet™/ DNA complex drop-wise onto the medium in each well and homogenize the mixture by gently swirling the plate.
- Remove DNA/GenJet™ complex-containing medium and replace with fresh complete serum/antibiotics containing medium ~5 hours post transfection.
- Check transfection efficiency 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