SL100489-HUVEC Store at 4 °C

GenJet™ In Vitro DNA Transfection Kit for HUVEC (Ver. II)

----- A Standard Protocol for Transfecting HUVEC

- ☐ 100 µl
- ☐ 500 µl
- ☐ 1000 µl

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

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. In combination of a proprietary transfection toxicity removal cocktail, GenJet™ In Vitro Transfection Kit (Ver. II) for HUVEC is pre-optimized and pre-conditioned for maximally transfecting HUVEC cells without visible cell death.

Contents Per Kit:

1. 1 x 1.0 ml of GenJet™ DNA Transfection Reagent for HUVEC (Ver. II)
2. 1 x 8.0 ml (5x) of GenJet™ Transfection Buffer

Procedures for Transfecting HUVEC:

Step I. Preparation of Working Solution of GenJet™ Transfection Buffer

GenJet™ Transfection Buffer (5x) is provided as 5 times concentrated stock solution. To make working solution, dilute one part of the stock solution with 4 parts of ddH_2O. The 1x GenJet™ Transfection Buffer is table at 4 °C~RT for 24 months.

Step 2. 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 ~70% 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 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

Step 3. Preparation of GenJet™-DNA Complex and Transfection Procedures

For HUVEC, the optimal ratio of GenJet™ (µL):DNA (µg) is 2.25:1. To ensure the optimal size of complex particles, we recommend using 1x GenJet™ Transfection Buffer to dilute DNA and GenJet™ Reagent.

The following protocol is given for transfection in 6-well plate, refer to **Table 2** for transfection in other culture formats. The optimal transfection conditions for HUVEC are given in the standard protocol described below.

Table 2. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Culture Medium (ml)	Plasmid DNA (µg)	Transfection Buffer (1x) (mL)	GenJet™ Reagent (µL)
96-well	0.1	0.1	0.010	0.225
48-well	0.25	0.25	0.025	0.5625
24-well	0.5	0.5	0.050	1.125
6-well	1	2.0	0.20	4.50
35 mm dish	1	2.0	0.20	4.50
60 mm dish	3	4.0	0.40	9.0
10 cm dish	6	10	1.0	22.5
T75 flask	6	11	1.1	24.75

- For each well, dilute 2.0 µg of DNA into 200 µl of 1xGenJet™ Transfection Buffer prepared from **Step 1**. Pipetting up and down to mix.
- Add 4.5 µl of GenJet™ reagent (Ver. II) into the diluted plasmid DNA. Vortex briefly to mix.
- Incubate for ~10 min at room temperature to allow GenJet™-DNA complexes to form. **Note:** Never keep GenJet™-DNA complexes longer than 20 minutes.
- Add the 200 µl GenJet™/ DNA complex drop-wise onto the cell culture and homogenize the mixture by gently swirling the plate.
- Remove DNA/GenJet™ complex-containing medium after overnight incubation followed by addition of complete serum/antibiotics containing medium.
- Check transfection efficiency 24 to 48 hours post transfection.

Storage: GenJet™ In Vitro Transfection Kit is stable for up to 12 months at 4 °C. This item shipped at ambient temperature