SKU # SL100489-HUVEC Store at 4 °C

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

----- A Protocol for Transfecting HUVEC

☐ 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 an upgraded version of the original GenJet™ In Vitro DNA Transfection Reagent. Through improved proprietary chemistry, the new formulation contains a higher density of DNA-condensing functional groups than the original GenJet™ reagent, resulting in 3- to 20-fold greater DNA delivery efficiency. GenJet™ (Ver. II) for HUVECs has been pre-optimized and pre-conditioned specifically for HUVECs. We provide a general protocol for transfecting HUVECs using a ~5.0 kb GFP expression plasmid as a reporter. Because transfection efficiency may vary with plasmid size and construct design, we recommend optimizing the transfection conditions for individual plasmids to achieve maximal transfection efficiency and transgene expression.

Contents Per Kit:

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

A General Protocol for Transfecting the HUVECs

Step I. Preparation of GenJet™ Transfection Buffer Working Solution (1x):

GenJet™ Transfection Buffer (5x) is provided as 5x concentrated stock solution. To make working solution (1x), dilute one part of the stock solution with 4 parts of ddH2O into a sterile bottle. The working solution is stable at RT for 24 months.

Note: *Always keep GenJet™ Transfection Buffer (5x) at RT. If refrigerated, white precipitates may appear. It won't affect the transfection efficiency. After dilution with 4 parts of ddH2O to make GenJet™ Transfection Buffer (1x) working solution, the white precipitates will disappear. Always keep GenJet™ Transfection Buffer working solution (1x) at RT.*

Step II. Cell Seeding

Seed cells 18–24 hours prior to transfection so that they reach approximately 80% confluency at the time of transfection. For optimal transfection performance, replace the culture medium with fresh complete growth medium containing serum and antibiotics approximately 60 minutes before transfection.

Table 1. Recommended Transfection Conditions for Various Culture Vessel Formats

Culture Dish	Culture Medium Volume (mL)	Plasmid DNA (µg)	GenJet™ Transfection Buffer (1x)	GenJet™ Reagent Ver. II (µL)
48 well plate	0.5	0.25	25	0.50
12 well plate	0.75	0.75	75	1.5
6-well plate	2.0	1.0	100	2.0
35 mm dish	2.0	1.0	100	2.0
60 mm dish	5	2.5	200	5.0
10 cm plate	10	5	400	10
T75 flask	10	5	400	10
15 cm plate	20	15	800	30

Step III. Preparation of GenJet™–DNA Complexes and Transfection Procedure

The optimal GenJet™ (µL) to DNA (µg) ratio is 2:1. The following protocol is provided for transfection in 24-well plates. For other culture formats, refer to **Table 1**. The optimal transfection conditions for HUVECs are described in the general protocol below.

- **Medium Preparation:** Aspirate existing medium from each well and replace with 1.0 mL of fresh complete medium containing serum and antibiotics 30–60 minutes prior to transfection.
- **DNA Dilution:** For each well, dilute 0.5 µg of plasmid DNA to 50 µL of GenJet™ Transfection Buffer (1x) in a 1.5 mL microcentrifuge tube. Gently mix immediately by pipetting up and down 3–4 times.
- **GenJet™ Addition:** Add 1.0 µL of GenJet™ reagent directly to the diluted DNA solution. Gently mix immediately by pipetting up and down 3–4 times.

  ⚠ ***Critical:*** *Do not use Opti-MEM as the diluent for either the DNA or GenJet™ reagent. Opti-MEM disrupts complex formation and significantly reduces transfection efficiency.*
- **Complex Formation:** Incubate the transfection mix at room temperature for 15–20 minutes to allow GenJet™–DNA complexes to form.

  ⚠ ***Critical:*** *Do not exceed 20 minutes of incubation. Prolonged incubation leads to aggregate formation and reduced transfection efficiency.*
- **Transfection:** Add the entire volume of GenJet™–DNA complex dropwise onto the cells in each well. Gently swirl the plate to ensure uniform distribution of the complex across the cell monolayer. Return the plate to the 37 ºC / 5% CO_2 incubator immediately.
- **Medium Replacement:** Approximately 18 hours post-transfection, aspirate the complex-containing medium and replace with fresh complete medium supplemented with serum and antibiotics.
- **Assessment of Transfection Efficiency:** Transfection efficiency should be evaluated 24–48 hours post-transfection

Storage: GenJet™ Reagent Ver. II is stable for 24 months at +4 °C. This item shipped at ambient temperature