Cat # SL100489-KER Store at 4 °C

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

----- A Protocol for Transfecting Primary Keratinocytes

- ☐ 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 Tranfection Reagent (Ver. II) is upgraded from GenJet™ In Vitro DNA Tranfection 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 terms of DNA delivery. GenJet™ (Ver. II) for primary keratinocytes was pre-optimized and conditioned for transfecting primary keratinocytes.

Procedures for Transfecting Primary keratinocytes:

Step A. 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

Table 2. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Transfection Volume (ml)	Plasmid DNA (µg)	Diluent Volume (mL)	GenJet™ Reagent (µL)
96-well	0.2	0.2	0.02	0.6
48-well	0.3	0.5	0.04	1
24-well	0.5	1.0	0.10	3
6-well	1.0	2	0.20	6
35 mm dish	1.0	2	0.20	6
60 mm dish	3	5	0.50	15
10 cm dish	5	6	1.00	18
T75 flask	7	10	1.30	30
250 ml flask	20	33	2.50	99

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

For transfecting primary keratinocytes, 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 primary keratinocytes 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 100 µL of serum-free DMEM with High Glucose. Vortex gently to mix.
- For each well, add 3 µL of GenJet™ reagent (Ver. II) into the above DMEM medium containing 1 µg of plasmid DNA. Vortex gently to mix.
  Note: Never use Opti-MEM to dilute GenJet™ reagent and DNA, it will disrupt transfection complex.
- Incubate the transfection mix for 15 min at room temperature to allow GenJet™-DNA complexes to form.
  Note: Never keep the DNA/GenJet™ complex longer than 20 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 next day post transfection.
- Check transfection efficiency 24 to 72 hours post transfection.

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