SL100489-KERA Store at 4 °C

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

----- A Protocol for Transfecting Keratinocytes

- ☐ 100 µl
- ☐ 500 µl
- ☐ 1000 µl

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Introduction:

GenJet™ In Vitro DNA Tranfection Reagent (Ver. II) is upgraded version of 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 DAN delivery. In combination of a proprietary transfection toxicity removal cocktail, GenJet™ In Vitro Transfection Kit (Ver. II) for keratinocytes is pre-optimized and pre-conditioned for maximally transfecting primary and immortalized keratinocytes with minimal cell death.

Contents Per Kit:

1. 1x1.0 ml of GenJet™ DNA Transfection Reagent (Ver. II)
2. 1x1.0 ml (x50) of HapiCell™ Transfection Toxicity Removal Cocktail

Procedures for Transfecting Keratinocytes:

Step 1. Preparation of HapiCell™ Transfection Toxicity Removal Cocktail Working Solution

HapiCell™ Transfection Toxicity Removal Cocktail is provided as 50x stock solution. Before performing transfection, prepare working solution by mixing the provided 1.0 ml stock solution with 49.0 ml of PBS (without calcium and magnesium) in a sterile bottle. The working solution is stable for years under storage at 4 °C and should be left at room temperature two hours before use.

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 ~90% 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 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 keratinocytes are given in the standard protocol described below.

Table 2. Recommended Amounts for Different Culture Vessel Formats

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

- 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.
  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 GenJet™-DNA complexes 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.
- Remove DNA/GenJet™ complex-containing medium 5 hours after transfection, briefly rinse keratinocytes with PBS followed by 2 minutes incubation with HapiCell™ Transfection Toxicity Removal Working Solution at RT with gentle shaking. Aspirate the toxicity removal working solution, briefly rinse again with PBS followed by addition of complete serum/antibiotics containing medium.
- Check transfection efficiency 24 to 48 hours post transfection.