Excellgen

293Expresso Transfection Reagent

Catalog #: EG-1089 **Store at 4 oC or -20 ºC**

Introduction

293Expresso in vitro DNA Tranfection Reagent contains a patent-pending blend of new gene delivery compounds, which greatly facilitate transfection of DNA to HEK293 cell lines such as HEK293T, HEK293FT, HEK293E, HEK293A.

Important Guidelines

-Add 1000 µl serum-free culture medium or 10 mM HEPES buffer, pH 7.2 aseptically before use. Store at -20 ºC or 4 ºC.

- In order to achieve higher efficiency, transfect cells at high density. 90~95% confluency is recommended

- To minimize cytotoxicity, transfect cells in presence of serum (10%) and antibiotics

- Change medium with serum (10%) and antibiotics 5 hours post transfection is optional

Procedures for Transfecting Mammalian Cells:

1. For Adherent Cells

Cell Seeding

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal 90~95% confluency at the time of transfection. Freshly complete culture medium with serum and antibiotics is added to each well 30~60 minutes before transfection.

Note: High serum levels (>5%) with antibiotics usually do not have inhibitory effect on transfection efficiency. For some cell lines, higher transfection efficiencies are observed in the presence of serum and antibiotics. We recommend you use complete medium containing serum and antibiotics initially.

Preparation of 293Expresso-DNA Complex and Transfection Procedures

For different cell types, the optimal ratio of GEM (µL):DNA (µg) varies from 1:1 to 3:1. We recommend the 293Expresso (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 293Expresso Reagent.

The following protocol is given for transfection in 24-well plates, refer to Table 1 for transfection in other culture formats. The optimal transfection conditions for a majority of adherent cell lines are given in the standard protocol described below.

Table 1. Recommended Amounts for Different Culture Vessel.

Culture Dish	Volume of medium (ml)	Amount of DNA (µg)	Diluent Volume (µL)	GeneExpresso (µL)
96-well plate	0.2	0.2	2 x 10	0.6
48-well plate	0.5	0.5	2 x 20	1.5
24-well plate	0.8	1.0	2 x 50	3
12-well plate	1	1.5	2 x 100	4.5
6-well plate	2	3	2 x 150	6
35 mm dish	2	3	2 x 100	9
60 mm dish	3	5	2 x 250	15
100 mm dish	6	8	2 x 500	24
T75 flask	10	18 - 36	2 x 750	54 - 108

-Add 1 µg of DNA into 50 µl of serum-free DMEM with High Glucose. Vortex gently and spin briefly to bring drops to bottom of the tube.

-Add 3 µl of 293Expresso reagent into 50 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.

-Immediately add the diluted 293Expresso Reagent to the diluted DNA solution.

- Vortex the solution immediately. Spin down briefly to bring liquid drops to bottom of the tube.

-Leave the solution **undisturbed** for 15-20 min at room temperature to allow 293Expresso-DNA complexes to form.

Note: Never keep the DNA-293Expresso complex longer than 20 minutes.

-Add 100 µl 293Expresso-DNA complex drop-wise into each well containing cells and medium. Mix gently by rocking the plate back and forth.

-Change medium 48 hours post transfection.

For sensitive cells, to lower cytotoxicity, remove 293Expresso-DNA complex and replace with complete medium 5 or 24 hours after transfection.

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Check transfection efficiency 24 to 48 hours post transfection.

For Suspension Cells

The following protocol is given for transfection in 6-well plate. The protocol can be scalded up or down according to culture volume.

Cell Seeding: Suspension cells are typically seeded the day of the transfection at a density of 0.5~1.0 x 10^6 cells per ml of culture. For optimal transfection conditions with 293Expresso, seed the number of cells adapted to the culture vessel format according to Table 2.

Table 2. Recommended Number of Suspension Cells to Seed

Culture Dishes	Number of Cells to Seed
100 mm Dish	5 x 10^6 – 1 x 10^7
60 mm Dish	2 x 10^6 – 5 x 10^6
35 mm Dish	5 x 10^5 – 2 x 10^6
6-well Plate	2 x 10^5 - 5 x 10^5
24-well Plate	1 x 10^5– 2 x 10^5
48-well Plate	5 x 10^4– 1 x 10^5
96-well Plate	2 x 10^4– 5 x 10^4

-30~60 minutes before transfection, warm fresh medium in a 37 ◦C water bath. Aspirate out the old medium from each well and add 0.5 ml fresh medium with serum and antibiotics.

293Expresso-DNA Complex Preparation and Transfection Procedures

For different cell types, the optimal ratio of 293Expresso (µL):DNA (µg) varies from 2:1 to 3:1. We recommend the 293Expresso (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 293Expresso reagent.

The following protocol is given for transfection in 6-well plates.

-For each well, dilute 2 µg of DNA into 100 µl of DMEM Serum-free Medium with High Glucose. Vortex gently and spin down briefly.

-For each well, dilute 6 µl of 293Expresso reagent into 100 µl of DMEM Serum-free Medium with High Glucose. Vortex gently and spin down briefly.

-Add the 100 µl 293Expresso solution immediately to the 100 µl 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 the bottom of the tube.

-Incubate for 15~20 minutes at room temperature.

-Add the 200 µl 293Expresso/ DNA mixture drop-wise onto the serum-containing medium in each well, homogenize the mixture by gently swirling the plate.

-Incubate at 37 ºC and 5% CO_2 in a humidified atmosphere.

-Transfection experiments are usually stopped after 24 to 48 hours and gene activity assessed. Cells growing in suspension are collected by centrifugation at 800 x g and then resuspended in the desired medium or buffer.

Storage: 293Expresso DNA In Vitro Transfection Reagent is stable for up to 24 months at -20 ºC, 3 months at 4 ºC. This product shipped at ambient temperature.

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