Excellgen

GeneExpresso Plus (GEP) Transfection Reagent

Catalog #: EG-1032 **Store at -20 ºC**

Introduction

GeneExpresso™ Plus DNA In Vitro Tranfection Reagent is enhanced version of GeneExpresso™ DNA In Vitro Transfection Reagent. GeneExpresso™ Plus is formulated by addition of a booster peptide, giving rise to up to 20 times higher efficiency on some mammalian cells in comparison of its previous version. GeneExpresso™ Plus was shown to efficiently deliver genes to various established cell lines as well as primary cells including HEK293, 293T, 293E, CHO, COS1, HeLa, NIH 3T3, insect cell lines (Sf9 and Sf21) and a variety of other eucaryotic cell lines with less toxicity. GeneExpresso™ Plus reagent, 1.0 ml, is sufficient for 300 to 600 transfections in 24 well plates or 150 to 300 transfections in 6 well plates.

Advantage

- Cell-dependent 3~20 times higher efficiency
- Top choice for hard-to-transfect cells
- Equally good for very long DNAs (up to 2.0 mb)
- Efficiency boosted in the presence of serum and antibiotics for most of cell types
- Exceptional high levels of recombinant protein production

Important Guidelines

-Add 1000 µl serum-free culture medium such as DMEM 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 GEP-DNA Complex and Transfection Procedures

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

15601 Crabbs Branch Way, Rockville, MD 20855-27438. USA ▪ Phone: 855-278-2398

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

-Immediately add the diluted GEP Reagent to the diluted DNA solution.

(**Important:** do not mix the solutions in the reverse order !)

- 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 GEP-DNA complexes to form.

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

-Add 100 µl GEP-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 GEP-DNA complex and replace with complete medium 5 or 24 hours after transfection.

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 GEP, 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.

GEP-DNA Complex Preparation and Transfection Procedures

For different cell types, the optimal ratio of GEP (µL):DNA (µg) varies from 2:1 to 3:1. We recommend the GEP (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 GEP 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 GEP reagent into 100 µl of DMEM Serum-free Medium with High Glucose. Vortex gently and spin down briefly.

-Add the 100 µl GEP 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 GEP/ 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: GEP 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.