GeneExpresso™ Plus In Vitro DNA Transfection Reagent

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Catalog #: IV-1032 **Store at 4 °C**

Introduction

GeneExpresso™ Plus In Vitro DNA Tranfection Reagent contains a patent-pending blend of new gene delivery compounds and a booster peptide, giving rise to up to 20 times higher efficiency on variety of mammalian cells in comparison of its previous version, GeneExpresso™.. 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.

Features:

-Cell-dependent 3~20 times higher efficiency than GeneExpresso™

-Top choice for hard-to-transfect cells

-Efficient 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

Plasmid DNA Transfection Protocol

Use the following procedure to transfect DNA into mammalian cells in a 24-well format. For other formats, see Table 1. All amounts and volumes are given on a per well basis. Prepare complexes using a DNA (µg) to GeneExpresso™ Plus (µl) ratio of 1:2 to 1:3 for most cell lines. Transfect cells at high cell density for high efficiency, high expression levels, and to minimize cytotoxicity. Optimization may be necessary (see Optimizing Plasmid DNA Transfection).

General Procedures for Transfecting Mammalian Cells:

Step I. 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. Complete culture medium with serum and antibiotics is freshly 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 specific cells, maximal transfection efficiencies are observed in the presence of serum and antibiotics. We recommend using complete serum/antibiotics-containing medium initially.

Step II. Preparation of GeneExpresso™ Plus-DNA Complex and Transfection Procedures

For different cell types, the optimal ratio of GeneExpresso™ Plus (µL):DNA (µg) varies from 2:1 to 3:1. We recommend the GeneExpresso™ Plus (µL):DNA (µg) ratio of 3:1 as a starting point which usually gives satisfactory transfection efficiency. If significant cytotoxicty is found, try GeneExpresso™ Plus (µl):DNA (µg) ratio to 2:1. To ensure the optimal size of complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and GeneExpresso™ Plus Reagent.

Table 1. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Transfection Volume (ml)	Plasmid DNA (µg)	Diluent Volume (µL)	GeneExpresso™ Plus (µL)
96-well plate	0.2	0.2	2 x 10	0.6
48-well plate	0.3	0.5	2 x 20	1
24-well plate	0.5	1	2 x 50	3
6-well plate	1.5	2	2 x 100	6
35 mm dish	1.5	2	2 x 100	6
60 mm dish	3	5	2 x 250	15
100 mm dish	8	7-8	2 x 500	21-24
T75 flask	10	18-36	2 x 750	54-108

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, as well as a general starting point for optimization are given in the standard protocol described below:

- For each well, add 0.5 ml of complete medium with serum and antibiotics freshly 30~60 minutes before transfection.

- For each well, dilute 1 µg of DNA into 50 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly to bring drops to the bottom of the tube.

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- For each well, dilute 3 µl of GeneExpresso™ Plus reagent into 50 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly to bring drops to the bottom of the tube.

- Add the diluted GeneExpresso™ 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 15~20 minutes at room temperature to allow GeneExpresso™-DNA complexes to form.

Note: Never keep the DNA/GeneExpresso™ Plus complex longer than 20 minutes

- Add the 100 µl GeneExpresso™ Plus/ DNA complex dropwise onto the medium in each well and homogenize the mixture by gently swirling the plate.

- Remove DNA/GeneExpresso™ Plus complex and replace with complete serum/antibiotics containing medium 12~18 hours post transfection. For sensitive cells, to lower cytotoxicity, remove GeneExpresso™ Plus/DNA complex and replace with complete medium 5 hours after transfection.

- Check transfection efficiency 24 to 48 hours post transfection.

Storage: GeneExpresso™ Plus DNA In Vitro Transfection Reagent should be stored at +4 °C. It should not be frozen. This product shipped at ambient temperature or blue ice.

Stability: GeneExpresso™ Plus DNA In Vitro Transfection Reagent is guaranteed for 6 months at +4 °C.

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Transfecting Hard-To-Transfect Mammalian Cells

Important Guidelines

- For high efficiency, transfect cells at high density. 90~95% confluency is highly recommended.

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

- Change medium with serum

Step I. Cell Culture

Cells should be plated at least 24 hours prior to transfection so that the monolayer cell density reaches to the optimal 95~100% confluency at the day of transfection.

Table 2. A Guideline for Optimal Cell Number Per Well in Different Culture Formats

Culture Dishes	Surface Area (cm2)	Number of Cells to Seed
T75 Flask	75	9.6 $x10^6$
100 mm Dish	58	7.3 $x10^6$
60 mm Dish	21	2.7 $x10^6$
35 mm Dish	9.6	1.2 $x10^6$
6-well Plate	9.6	1.2 $x10^6$
12-well Plate	3.5	0.44 $x10^6$
24-well Plate	1.9	0.24 $x10^6$
48-well Plate	1.0	0.11 $x10^6$
96-well Plate	0.3	0.31 $x10^5$

Table 3. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Transfection Volume (ml)	Plasmid DNA (μg)	GeneExpresso™ Plus (μL)
96-well plate	0.02	0.2	0.6
48-well plate	0.04	0.5	1
24-well plate	0.1	1	3
12-well plate	0.2	2	6
6-well plate	0.2	2	6
60 mm dish	0.5	5	15
100 mm dish	1.0	8	24
T75 Flask	1.5	36	108
250 ml flask	2.5	100	300

Step II. Preparation of Cells in Suspension

The following protocol is given for transfecting hard-to-transfect mammalian cells in 6-well plates, refer to **Table 2** for optimal cell number per well per culture vessels' surface area. The optimal transfection conditions for mammalian cells are given in the standard protocol described below.

- Detach the cells with trypsin/EDTA and stop the trypsinization with complete culture medium.

Note: Cells that are difficult to detach may be placed at 37 °C for 5~15 min to facilitate detachment

- Take an aliquot of trypsinized cell suspension and count the cells to determine the cell density.

- Centrifuge the required $1.2x10^6$ cells per well for 6-well plate at 150xg at room temperature for 10 min.

- Use fine tip pipette to remove supernatant completely so that no residual medium covers the cell pellet.

Step III. Preparation and Application of Transfection Complex

For hard-to-transfect mammalian cells, the optimal ratio of GeneExpresso™ Plus (μL):DNA (μg) is 4:1. To ensure the optimal size of complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and GeneExpresso™ Plus Reagent.

The following protocol is given for transfection in 6-well plates, refer to **Table 3** for transfection in other culture formats.

- For each well of 6-well plate, dilute 2 μg of DNA into 100 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 of 6-well plate, dilute 8 μl of GeneExpresso™ Plus reagent into 100 μl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.

- Add the diluted GeneExpresso™ Plus Reagent immediately to the diluted DNA solution all at once.

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- Vortex-mix the solution immediately and spin down briefly to bring drops to bottom of the tube followed by incubation of 10 minutes at room temperature to allow transfection complexes to form.

Important: Never keep the transfection complexes longer than 15 minutes

- Gently resuspend the cell pellet prepared from **Step II** immediately in the 200 µl transfection complex and incubate at 37 °C for 20 minutes.

- At the end of incubation, add 2.0 ml of pre-warmed fresh complete cell growth medium to cells and plate onto one well of a 6-well plate.

- Remove transfection complex containing medium gently and refill with complete culture medium 5~12 hours after plating.

- Check transfection efficiency 24 to 48 hours post transfection.

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Protocol for generation of Lentivirus from 293T cell

Important Transfection Guidelines:

- For high efficiency, transfect cells at high density. 90~95% confluency is highly recommended

- To lower 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 293T Cells:

Cell Seeding (see Table 4):

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal ~95% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly 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 specific 293 cells, maximal transfection efficiencies are observed in the presence of serum and antibiotics. We recommend using complete serum/antibiotics-containing medium initially.

Table 4. A Guideline for Optimal Cell Number Per Well in Different Culture Formats

Culture Dishes	Surface Area (cm2)	Number of Cells to Seed
T75 Flask	75	$3.0 - 6.0 \times 10^6$
100 mm Dish	58	$2.2 - 4.4 \times 10^6$
60 mm Dish	21	$0.9 - 1.8 \times 10^6$
35 mm Dish	9.6	$3.5 - 7.0 \times 10^5$
6-well Plate	9.6	$4.0 - 8.0 \times 10^5$
12-well Plate	3.5	$1.5 - 3.0 \times 10^5$
24-well Plate	1.9	$0.8 - 1.6 \times 10^5$
48-well Plate	1.0	$4.0 - 8.0 \times 10^4$
96-well Plate	0.3	$1.2 - 2.4 \times 10^4$

Preparation of GeneExpresso™ Plus-DNA Complex and Transfection Procedures

The following protocol is given for transfection in 10 cm dish. For other culture formats, scale up or down per culture dish's surface. The optimal transfection conditions are given in the standard protocol described below.

-Cell confluency should be ~95 % at the day of transfection

- For each 10 cm dish, add 5.0 ml of complete medium with serum and antibiotics freshly 30~60 minutes before transfection.

- For each dish, dilute total 45 µg of DNA (three DNAs with 15 µg each) into 500 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly to bring drops to bottom of the tube.

- For each dish, dilute 60 µl of GeneExpresso™ Plus reagent into 500 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.

- Add the diluted GeneExpresso™ Plus 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 15~20 minutes at room temperature to allow GeneExpresso™ Plus-DNA complexes to form.

Note: Never keep the DNA/GeneExpresso™ Plus complex longer than 20 minutes

- Add the 1000 µl GeneExpresso™ Plus / DNA complex dropwise onto the medium in each dish and homogenize the mixture by gently swirling the plate.

- Remove DNA/GeneExpresso™ Plus complex-containing medium and replace with fresh complete serum/antibiotics containing medium 5 hours post transfection.

- Check transfection efficiency and virus titer 24 to 48 hours post transfection. 48 hours gives better titers.

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Procedures for Transfecting Bacmids into Sf9 Cells:

1. Count Sf9 cells, and adjust cell density to 5 x10^5 cells/ml in unsupplemented SF900II media

2. Seed 2 ml of cell suspension per well (1 x 10^6 cells/well).

3. Label 2 wells as "negative control", 2 wells as "1 µg DNA", and 2 wells as "2 µg DNA"

4. Incubate dishes at 27°C for 30-60 minutes (enough time to allow the cells to attach to the bottom of the wells).

5. Aliquot 500 µl of sterile diluent (150 mM NaCl) into three 1.5 ml Eppendorf tubes. Label the tubes "0", "1 µg", and "2 µg".

These will serve as 2.5X Master Mixes for each of the three conditions.

NOTE: The sterile diluent should be 150 mM NaCl which is essential for DNA/GeneExpresso™ Plus complex formation.

IT IS IMPORTANT THAT THE DNA IS ADDED FIRST AND THE GeneExpresso™ PLUS Reagent IS ADDED SECOND TO EACH TUBE.

6. Aliquot 2.5 µg of bacmid into the "1 µg" Master Mix tube.

7. Aliquot 5 µg of bacmid into the "2 µg" Master Mix tube.

8. Briefly vortex the tubes.

9. To the "1 µg" Master Mix tube, add 10 µl of GeneExpresso™ Plus Reagent and *IMMEDIATELY VORTEX* for 5 seconds.

10. To the "2 µg" Master Mix tube, add 20 µl of GeneExpresso™ Reagent and *IMMEDIATELY VORTEX* for 5 seconds.

11. Allow the Master Mix tubes to sit in the hood for 10~15 minutes.

12. During the 10~15 minutes incubation period, remove the freshly seeded plates from the incubator. Remove the media from each well, and wash adherent cell monolayer 1X with 2 ml of unsupplemented SF900II media.

13. Add 2 ml of SF900II + gentamicin to each well.

14. After the 10-15 minutes incubation, mix the contents of each Master Mix via gentle pipetting (*DO NOT REVORTEX*).

15. Add 200 µl of each Master Mix to the appropriate well, and mix by gently rocking the plate(s).

16. Place plate(s) on a level surface at 27°C

17. Harvest supernatants at day 5 post-transfection, for use in high titer stock production.

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