

Introduction

GeneExpresso™ in vitro DNA Transfection Reagent contains a patent-pending blend of new gene delivery compounds, which greatly facilitate transfection of DNA to various established cell lines as well as primary cells.

Important Guidelines

- Add 1ml serum-free cell culture medium or 10 mM HEPES, pH 7.2~7.4 buffer aseptically.

- 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 GeneExpresso™-DNA Complex and Transfection Procedures

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

-Add 1 μg of DNA into 50 μl of serum-free DMEM

Culture Dish	Transfection Volume (ml)	Plasmid DNA (μg)	Diluent Volume (μL)	GeneExpresso (μL)
24-well plate	0.5	0.5	2 x 25	1.5
12-well plate	1	1	2 x 50	3
6-well plate	2	2	2 x 100	6
100 mm dish	8	7 - 8	2 x 500	21 - 24
T75 flask	10	18 - 36	2 x 750	54 - 108
150 mm dish	25	25 - 40 μg	2 x 1500	75 -120

with High Glucose. Vortex gently and spin briefly to bring drops to bottom of the tube.

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

-Immediately add the diluted GeneExpresso™ 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 GeneExpresso™-DNA complexes to form.

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

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

-Change medium 12~18 hours post transfection.

For sensitive cells, to lower cytotoxicity, remove GeneExpresso™-DNA complex and replace with complete medium 5 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 scaled up or down according to culture volume.

Cell Seeding: Suspension cells are typically seeded the day of the transfection at a density of $0.5 \sim 1.0 \times 10^6$ cells per ml of culture. For optimal transfection conditions with GeneExpresso™, 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	$5 \times 10^6 - 1 \times 10^7$
60 mm Dish	$2 \times 10^6 - 5 \times 10^6$
35 mm Dish	$5 \times 10^5 - 2 \times 10^6$
6-well Plate	$2 \times 10^5 - 5 \times 10^5$
24-well Plate	$1 \times 10^5 - 2 \times 10^5$
48-well Plate	$5 \times 10^4 - 1 \times 10^5$
96-well Plate	$2 \times 10^4 - 5 \times 10^4$

-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.

GeneExpresso™-DNA Complex Preparation and Transfection Procedures

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

-Add the 100 μl GeneExpresso™ 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 GeneExpresso™/ DNA mixture drop-wise onto the serum-containing medium in each well, homogenize the mixture by gently swirling the plate.

-Incubate at 37 °C and 5% CO₂ 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: GeneExpresso™ DNA In Vitro Transfection Reagent is stable for up to 12 months at +4 °C. This product shipped at ambient temperature.

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

Culture Dishes	Surface Area (cm ²)	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 4. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Transfection Volume (ml)	Plasmid DNA (μg)	GeneExpresso (μL)
96-well plate	0.02	0.2	0.8
48-well plate	0.04	0.5	2
24-well plate	0.1	1	4
12-well plate	0.2	2	8
6-well plate	0.2	2	8
60 mm dish	0.5	5	20
100 mm dish	1.0	8	32
T75 Flask	1.5	36	144
250 ml flask	2.5	100	400

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 3** 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 1.2x10⁶ 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™ (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™ Reagent.

The following protocol is given for transfection in 6-well plates, refer to **Table 4** 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™ reagent into 100 μl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.

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

-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.

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 5):

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

Culture Dishes	Surface Area (cm ²)	Number of Cells to Seed
T75 Flask	75	3.0 - 6.0 x10 ⁶
100 mm Dish	58	2.2 - 4.4 x10 ⁶

60 mm Dish	21	0.9 - 1.8 x10 ⁶
35 mm Dish	9.6	3.5 - 7.0 x10 ⁵
6-well Plate	9.6	4.0 -8.0 x10 ⁵
12-well Plate	3.5	1.5 - 3.0 x10 ⁵
24-well Plate	1.9	0.8 - 1.6 x10 ⁵
48-well Plate	1.0	4.0 - 8.0 x10 ⁴
96-well Plate	0.3	1.2 - 2.4 x10 ⁴

Preparation of GeneExpresso™-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™ reagent into 500 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.

-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™ complex longer than 20 minutes

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

- Remove DNA/GeneExpresso™ 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.

Procedures for Transfecting Bacmids into Sf9 Cells:

1. Count Sf9 cells, and adjust cell density to 5×10^5 cells/ml in unsupplemented SF900II media
2. Seed 2 ml of cell suspension per well (1×10^6 cells/well).
3. Label 2 wells as “negative control”, 2 wells as “1 μ g DNA”, and 2 wells as “2 μ g 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 μ l of sterile diluent (150 mM NaCl) into three 1.5 ml Eppendorf tubes. Label the tubes “0”, “1 μ g”, and “2 μ g”.

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™ complex formation.

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

6. Aliquot 2.5 μ g of bacmid into the “1 μ g” Master Mix tube.
7. Aliquot 5 μ g of bacmid into the “2 μ g” Master Mix tube.
8. Briefly vortex the tubes.
9. To the “1 μ g” Master Mix tube, add 10 μ l of GeneExpresso™ Reagent and ***IMMEDIATELY VORTEX*** for 5 seconds.
10. To the “2 μ g” Master Mix tube, add 20 μ l of GeneExpresso™ Reagent and ***IMMEDIATELY VORTEX*** for 5 seconds.
11. Allow the Master Mix tubes to sit in the hood for 10~15 minutes.