

Introduction

GeneExpresso™ 8000 DNA In Vitro Transfection Reagent is formulated with proprietary cationic polymer-lipid conjugate to ensure that the transfection protocol is identical to Lipofectamine™ 2000. GeneExpresso™ 8000 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™ 8000 reagent, 1.0 ml, is sufficient for 300 to 600 transfections in 24 well plates or 150 to 300 transfections in 6 well plates.

- No need to change SOP if you are currently using Lipofectamine 2000.
- No serum interference: DNA-GeneExpresso™ 8000 complexes can be added directly to cells in culture medium, in the presence or absence of serum.
- No need to change medium after transfection, but the DNA-GeneExpresso 8000 complexes can be removed after 4-6 hours.
- Can be used to transfect siRNA into mammalian cells
- Can be used to cotransfect plasmid DNA and RNAi into mammalian cells

Important Guidelines

- Invitrogen Opti-MEM® I Reduced Serum Medium (Cat. No. 31985-062) is recommended for diluting GeneExpresso™ 8000 and nucleic acids before complexing.
- Do not add antibiotics to media during transfection as this causes cell death.
- Maintain the same seeding conditions between experiments.
- Test serum-free media for compatibility with GeneExpresso™ 8000 since some serum-free formulations (e.g. CD293, SFM II, VP-SFM) may inhibit GeneExpresso™ 8000-mediated transfection.

Procedures for Transfecting Plasmid DNA into Mammalian Cells

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™ 8000 (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).

1. Adherent cells: One day before transfection, plate 0.5-2 x 10⁵ cells in 500 µl of growth medium without antibiotics so that cells will be 90-95% confluent at the time of transfection.

Suspension cells: Just prior to preparing complexes, plate 4-8 x 10⁵ cells in 500 µl of growth medium without antibiotics.

2. For each transfection sample, prepare complexes as follows:

a. Dilute DNA in 50 µl of Opti-MEM® I Reduced Serum Medium without serum (or other medium without serum). Mix gently.

b. Mix GeneExpresso™ 8000 gently before use, then dilute the appropriate amount in 50 µl of Opti-MEM® I Medium. Incubate for 5 minutes at room temperature. Note: Proceed to Step c within 25 minutes.

c. After the 5 minute incubation, combine the diluted DNA with diluted GeneExpresso™ 8000 (total volume = 100 µl). Mix gently and incubate for 20 minutes at room temperature (solution may appear cloudy). Note: Complexes are stable for 6 hours at room temperature.

3. Add the 100 µl of complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth.

4. Incubate cells at 37°C in a CO₂ incubator for 18-48 hours prior to testing for transgene expression. Medium may be changed after 4-6 hours.

5. For stable cell lines: Passage cells at a 1:10 (or higher dilution) into fresh growth medium 24 hours after transfection. Add selective medium (if desired) the following day.

Optimizing Plasmid DNA Transfection

To obtain the highest transfection efficiency and low cytotoxicity, optimize transfection conditions by varying cell density as well as DNA and GeneExpresso™ 8000 concentrations. Make sure that

GeneExpresso™ 8000 in vitro DNA Transfection Reagent

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cells are greater than 90% confluent and vary DNA(μg):GeneExpresso™ 8000 (μl) ratios from 1:0.5 to 1:5.

Scaling Up or Down Transfections

To transfect cells in different tissue culture formats, vary the amounts of GeneExpresso™ 8000, nucleic acid, cells, and medium used in proportion to the relative surface area, as shown in the table. With automated, high-throughput systems, a complexing volume of 50 μl is recommended for transfections in 96-well plates. Note: You may perform rapid 96-well

plate transfections by plating cells directly into the transfection mix. Prepare complexes in the plate and directly add cells at twice the cell density as in the basic protocol in a 100 μl volume. Cells will adhere as usual in the presence of complexes.

Table 1, Recommended Amounts for Different Culture Vessel when Transfecting DNA

Culture Dish	Transfection Volume (ml)	Plasmid DNA (μg)	Diluent Volume (μL)	GeneExpresso™ 8000 (μL)
96-well plate	0.1	0.2	2 x 25	0.5
24-well plate	0.5	0.8	2 x 50	2
12-well plate	1	1.6	2 x 100	4
6-well plate	1.5	4.0	2 x 250	10
60 mm dish	3	8	2 x 500	20
100 mm dish	6	24	2 x 1500	60

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Cotransfecting Plasmid DNA and RNAi into Mammalian Cells Using GeneExpresso 8000

Important Guidelines

- ❖ Transfect cells at 80-90% confluence.
- ❖ Do not add antibiotics to the medium during transfection as this reduces transfection efficiency and causes cell death.
- ❖ Use Opti-MEM® I Reduced Serum Medium to dilute GeneExpresso 8000, DNA, and dsRNA oligomers prior to complex formation.

Transfection Procedure

Use the following procedure to transfect DNA into mammalian cells in a 24-well format. For other formats, see Table 2. All amounts and volumes are given on a per well basis.

Table 2, Recommended Amounts for Different Culture Vessel when Transfecting siRNA

Culture Plate	Transfection Volume (µl)	Plasmid DNA (ng)	dsRNA (pmol)/RNAi vector (ng)	Diluent Volume (µL)	GeneExpresso™ 8000 (µL)
96-well plate	100	10-100	0.1-1 pmol/150-300 ng	25	0.2-0.5
24-well plate	500	100-200	1-10 pmol/300-600 ng	50	0.5-1.5
12-well plate	1000	200-400	2-20 pmol/600-1200 ng	100	1-3
6-well plate	2000	500-1000	5-50 pmol/1500-3000 ng	250	2.5-6

For example, for transfecting cells grown in 24-well plate, you can use 150 ng of plasmid DNA, 5 pmol of RNAi, and 1 µl of GeneExpresso 2000.

1. One day before transfection, plate cells in the appropriate amount of growth medium without antibiotics such that they will be 80-90% confluent at the time of transfection.

2. For each transfection sample, prepare DNA-RNAi-GeneExpresso 8000 complexes as follows.

a. Dilute the DNA and RNAi molecule in the appropriate amount of Opti-MEM® I Medium without serum. Mix gently.

b. Mix GeneExpresso 8000 gently before use, then dilute the appropriate amount in Opti-MEM® I Medium without serum. Mix gently and incubate for 5 minutes at room temperature.

c. After the 5 minute incubation, combine the diluted DNA and RNAi molecule with the diluted GeneExpresso 8000. Mix gently and incubate for 20 minutes at room temperature to allow formation of DNA-RNA-GeneExpresso 8000 complex.

3. Add the DNA-RNAi molecule-GeneExpresso 8000 complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth.

4. Incubate the cells at 37°C in a CO2 incubator. Harvest cells 24-48 hours after transfection. Removal of complexes or media change after 4-6 hours is optional.

Storage: GeneExpresso™ 8000 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™ 8000 DNA In Vitro Transfection Reagent is guaranteed for 12 months at +4 °C.