

# GeneExpresso™ 8000 In Vitro DNA Transfection Reagent



Catalog #: IV-1074      Store at 4 °C

## 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 10<sup>5</sup> 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 10<sup>5</sup> 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 CO<sub>2</sub> 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

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cytotoxicity, optimize transfection conditions by varying cell density as well as DNA and GeneExpresso™ 8000 concentrations. Make sure that 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

**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.