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Introduction

GeneTran is a lipid-based reagent designed and formulated with proprietary technology for transfecting DNA or siRNA into a variety of eukaryotic cell lines, including HEK293, CHO, COS, NIH3T3, MCF-7, PC12, RKO, C2C12 myoblasts, mouse embryonic stem cells (ES) and fibroblasts (MEF), mesenchymal stem cells, NCCIT, S2 (Schneider) Drosophila cells as well as primary cultures of human carcinoma, rat adrenal chromaffin cells, mouse and rat cortical neurons, hepatocytes, and etc. GeneTran also offers extremely high transfection efficiencies and minimal cytotoxicity for suspension cultures including HEK 293, mouse lymphocytes, and insect cells (sf9, sf21, High-Five). The reagent works very well in cultures with serum concentration range from 0 to 20% FBS.

Major Features

- Exceptional efficiency of transfection for a broad range of adherent cell types.
- Especially good for embryonic stem (ES) cells and difficult-to-transect primary cultures.
- Equally efficient for suspension cultures of HEK293, and sf9, sf21 and High 5 insect cells.
- Higher efficiency and lower toxicity than all commercially available transfection reagents currently on the market.
- Transfection efficiency not affected by serum. Works equally well in serum-containing (up to 20% FBS) or serum-free media.
- High levels of recombinant protein production at 24-72 hours after transfection.
- Works very well for both single DNA transfection and multi-DNA co-transfection.
- Low cost (Almost 50% price off compare to other major commercial products).

Storage and Stability

GeneTran should be stored sterile at 4 ℃（**not -20 ºC**）. It is stable for 12 months or longer from the date of production.

Kit Contents

Catalog#	GT1212-00	GT1212-01	GT1212-02	GT1212-03	GT1212-04
GeneTran reagent	100 µL	0.5 mL	1.0 mL	1.5 mL	6.0 mL
Reagent B	10µL	100 µL	200 µL	300 µL	1.2 mL

Important Notes

- **GeneTran** is a proprietary product of Biomiga, any material transfer without permission is prohibited.
- ***GT1212-00: Spin down the sample vial briefly before use.***

Plasmid DNA Transfection Protocols

Use the following procedure to transfect DNA into eukaryotic cells in a 35 mm dish or a 6-well plate. For other size of cell culture dish, refer to Table 1 for scaling up and down. All amounts and volumes are given on a per well basis. Optimization may be necessary (see Optimizing Plasmid DNA Transfection, Page 7).

- **Transfection of adherent cells (35 mm dishes or 6-well plate)**

Split the cells one day before transfection to reach 90-95% confluence in the day of transfection.

1. For each transfection sample, prepare the transfection complexes as follows:

   In a sterile 1.5 ml microtube, mix the following reagents:

   2.5 µg Plasmid DNA
   3 -6.5 µL GeneTran
   X µL Serum-free* DMEM (or any other serum free-medium, PBS, DPBS etc.)

   The total reaction is 100 µL.

   - **Absolutely no Opti-MEM and serum in the transfection mixture**

2. Pipetting up and down the mixture a few times. Spin briefly in a centrifuge. Leave the mixture at room temperature for 20-40 mins.

3. Add the entire mixture evenly to cells in the 35 mm culture dish or 6-well plate. Mix the reagent with the cells by tilting the dish or plate several times. Return the dish to a CO_2 incubator.

   Optional: For protein expression boost, add **1 µL of Reagent B** to the dish, tilting the dish to mix

   Note: Serum concentration in the growth medium has not effect on the transfection efficiency. Up to 20% of fetal bovine serum has been tested without significantly changing the transfection efficiency.

4. Incubate cells for 18-48 hours prior to testing for transgene expression. At any time between 4-6 hours after the transfection, replace the medium in the dish with 2 ml (or any volume you'd like) fresh growth medium. For transient protein expression, collect the medium in 4-5 days after transfection.

5. For stable cell lines: Passage cell at a 1:10 into fresh growth medium 24 hours after transfection.

- **Transfection of suspension cells (35 mm dishes or 6-well plate)**

The confluence should reach 90% in the day of transfection.

1. In a sterile 1.5 mL tube, mix the following reagents

2.5 µg	**Plasmid DNA**
3 -6.5 µL	**GeneTran**
X µL	**Serum-free* DMEM (or any other serum free-medium, PBS, DPBS etc.)**

The total reaction is 100 µL.

- **Absolutely no Opti-MEM and serum in the transfection mixture**

2. Pipetting up and down the mixture a few times. Spin briefly in a centrifuge. Leave the mixture at room temperature for 20-40 mins.

3. Add the entire mixture evenly to cells in the 35 mm culture dish or 6-well plate. Mix the reagent with the cells by tilting the dish or plate several times. Return the dish to a CO_2 incubator.

Optional: For protein expression boost, add **1 µL of Reagent B** to the dish, tilting the dish to mix

Note: Serum concentration in the growth medium has not effect on the transfection efficiency. Up to 20% of fetal bovine serum has been tested without significantly changing the transfection efficiency.

4. Incubate cells for 18-48 hours prior to testing for transgene expression. At any time between 4-6 hours after the transfection, replace the medium in the dish with 2 ml (or any volume you'd like) fresh growth medium. For transient protein expression, collect the medium in 4-5 days after transfection.

5. For stable cell lines: Passage cell at a 1:10 into fresh growth medium 24 hours after transfection.

Table 1. Scaling Up or Down Transfections (based on plating medium volume)

Culture dish	Surf. Area per well (cm^2)	Shared reagents		DNA transfection	
		Vol. Of Plating Medium	Vol. Of mixing Medium	DNA	GeneTran
10 cm	56	10 mL	500 µL	10 µg	15-20 µL
60 mm	21	3 mL	150 µL	3 µg	4.5-6 µL
35 mm	8	2 mL	100 µL	2.5 µg	3-5 µL
6-well	9.5	2 mL	100 µL	2.5 µg	3-6.5 µL
12-well	3.8	1 mL	50 µL	1 µg	1-2 µL
24-well	1.9	0.5 mL	25 µL	0.5 µg	0.8-1 µL
48-well	0.95	0.25 mL	12.5 µL	0.25 µg	0.25-0.5 µL
96-well	0.32	0.1 mL	5 µL	0.1 µg	0.1-0.2 µL

Optimization Procedure

- The protocol described above has been optimized for HEK293, COS, and other "easy to transfect" cell lines, which you can easily get >90% transfection efficiency.

- For C2C12 muscle cell line: which normally give <10% transfection efficiency with FuGENE-6 or Lipofectamine-2000, >70% transfection efficiency has been reached by using a mixture containing:

6 µg Plasmid DNA
10-12 µL GeneTran
X µL Serum-free* DMEM (or any other serum free-medium, PBS, DPBS etc.)

The total reaction is 100 µL.

- For other cell lines, you may need to optimize the condition by doing the following:

a. If you do not use DMEM for your cells, you can use any other serum-free medium, PBS, DPBS, to prepare the transfection complex.

b. Increase the amount of plasmid DNA to 3, 4, or 6 µg per 35 mm dish.

c. Increase the amount of GeneTran to up to 15 µl per 35 mm dish.

d. If you do get good transfection efficiency, but see lots of dead cells, you should reduce the amount of plasmid to 1 µg or lower. Also, you may want to reduce the time of incubation in GeneTran to 5 – 24 hours.

e. Use the following table to optimize the transfection:

Note	DNA	GeneTran
In case of cellular toxicity	Reduce to 1- 2 μg	Reduce to 1-2 μL
In case of low transfection efficiency by standard protocol	Increase to 4 μg for very difficult-to-transfect cells	Increase to > 1:3
	Increase to 6 μg for extremely difficult-to-transfect cells	Increase to > 1:3

Limited Use and Warranty

This product is intended for *in vitro* research use only. Not for use in human.

This product is warranted to perform as described in its labeling and in BEIWO's literature when used in accordance with instructions. No other warranties of any kind expressed or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by BEIWO. BEIWO's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of BEIWO, to replace the products, BEIWO shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or learn more product information, please contact us or visit our website.

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