Ver: 1904

High Efficiency Transfection Reagent

（BW-GT2211）

Contents

Kit Contents

Catalog#	BW-GT2211-00	BW-GT2211-01	BW-GT2211-02
Volume	20 μL	1.0 mL	10×1.0 mL
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Introduction

GeneTran™ III is a proprietary formulation for the transfection of nucleic acids (DNA and RNA) into eukaryotic cells providing the following advantages:

• Highest transfection efficiency in many cell types and formats (e.g. 96-well).

• Nucleic acid-GeneTran™ III complexes can be added directly to cells in culture medium, in the presence or absence of serum.

• It is not necessary to remove complexes or change/add medium after transfection, but complexes may be removed after 4-6 hours.

Storage and Stability

The kit should be stored at 4°C (**not -20°C**). It is stable for 12 months from the date of production.

Important Notes

• Do not add antibiotics to medium during transfection as this causes cell death.

• Maintain the same seeding conditions between experiments.

•Test serum-free medium for compatibility with GeneTran™ III since some serum-free formulations (e.g. CD293, SFM II, VP-SFM) may inhibit cationic lipid-mediated transfection.

siRNA Transfection Protocols

Use this brief procedure to transfect siRNA into mammalian cells in a 24-well format. For other formats, see Scaling Up or Down Transfections(see page **5**). All amounts and volumes are given on a per well basis. Use this procedure as a starting point, optimize transfections as described in Optimizing siRNA Transfection, especially if you are transfecting a mammalian cell line for the first time.

1. One day before transfection, plate cells in 500 µL of growth medium without antibiotics such that they will be 30-50% confluent at the time of transfection.

   Note: Transfecting cells at a lower density allow a longer interval between transfection and assay time, and minimize the loss of cell viability due to cell overgrowth.

2. For each transfection sample, prepare oligomer-GeneTran™ III complexes as follows:

   a. Dilute 20 pmol siRNA oligomer in 50 µL serum free-medium (final concentration of RNA when added to the cells is 33 nM). Mix gently.

   b. Mix **GeneTran™ III** gently before use, then dilute **1 µL** in 50 µL serum free-medium. Mix gently and incubate for 5 minutes at room temperature.

   Note: Proceed to the next step within 25 minutes.

   c. After the 5-minute incubation, combine the diluted oligomer with the diluted GeneTran™ III. Mix gently and incubate for 20 minutes at room temperature (solution may appear cloudy).

3. Add the oligomer-GeneTran™ III complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth.

   Incubate the cells at 37°C in a CO_2 incubator for 24-96 hours until you are ready to assay for gene knockdown. Medium may be changed after 4-6 hours.

Optimizing siRNA Transfection

To obtain the highest transfection efficiency and low non-specific effects, optimize transfection conditions by varying RNA and GeneTran™ III concentrations. Test 10-50 pmol RNA and 0.5-1.5 µL GeneTran™ III for 24-well format. Depending on the nature of the target gene, transfecting cells at higher densities may also be considered when optimizing conditions.

Plasmid DNA Transfection

Use the following procedure to transfect DNA into mammalian cells in a 24-well format. For other formats, (see Optimizing Plasmid DNA Transfection, page **5**). All amounts and volumes are given on a per well basis. Prepare complexes using a DNA (µg) to GeneTran™ III (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, page **5**).

1. **Adherent cells**: One day before transfection, plate 0.5-2 x 10^5 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 10^5 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 serum-free medium (or other medium without serum). Mix gently.

b. Mix **GeneTran™ III** gently before use, then dilute the **appropriate amount** in 50 µL of serum-free medium. Incubate for 5 minutes at room temperature.

Note: Proceed to the next step within 25 minutes.

c. After the 5 minute incubation, combine the diluted DNA with diluted GeneTran™ III (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_2 incubator for 18-48 hours prior to testing for gene 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 GeneTran™ III concentrations. Make sure that cells are greater than 90% confluent and vary DNA (µg): GeneTran™ III (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 GeneTran™ III , nucleic acid, cells, and medium used in proportion to the relative surface area, as shown in the table. For 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.

Culture vessel	Surf. area per well[1]	Shared reagents		DNA transfection		siRNA transfection	
		Vol of plating medium	Vol of dilution medium[2]	DNA	GeneTran™ III	RNA	GeneTran™ III
96-well	0.3 cm^2	100 µL	2 x 25 µL	0.2 µg	0.5 µL	5 pmol	0.25 µL

24-well	2 cm^2	500 µL	2 x 50 µL	0.8 µg	2.0 µL	20 pmol	1.0 µL
12-well	4 cm^2	1 mL	2 x 100 µL	1.6 µg	4.0 µL	40 pmol	2.0 µL
6-well	10 cm^2	2 mL	2 x 250 µL	4.0 µg	10 µL	100 pmol	5 µL
60-mm	20 cm^2	5 mL	2 x 0.5 mL	8.0 µg	20 µL	200 pmol	10 µL
10-cm	60 cm^2	15 mL	2 x 1.5 mL	24 µg	60 µL	600 pmol	30 µL

[1] Surface areas may vary depending on the manufacturer.

[2] Volumes of dilution medium in step **2a** & **2b** of DNA or siRNA transfection protocols.

Related Products

Catalog #	Product Name	Preps	Price (RMB)
GT1211-01	GeneTran™ High Efficiency Transfection Reagent	1.5 mL	1050.00
GT1211-02	GeneTran™ High Efficiency Transfection Reagent	1.0 mL	1850.00
GT1212-01	GeneTran™ Transfection Reagent For Transient Protein Expression	0.5 mL	1120.00
GT1212-02	GeneTran™ Transfection Reagent For Transient Protein Expression	1.0 mL	1970.00
BW-GT2211-01	GeneTran™ III High Efficiency Transfection Reagent	1.0 mL	1970.00
BW-GT2211-02	GeneTran™ III High Efficiency Transfection Reagent	10 mL	18000.00

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.

Contact Us: 400-115-2855

www.beiwobiomedical.com

Customer Support:

market@beiwobiomedical.com

Technical Support:

tech@beiwobiomedical.com