# Product Information

## LipoGeneOM 2000S Transfection Reagent

Catalog: OM625684

Unit Size: 0.75mL, 1.5mL

## Storage and Handling

Storage at 4℃. **Do not freeze**. Product is stable for at least 12 months from date of receipt when stored as recommended.

## Description

LipoGene™ 2000 Plus Transfection Reagent is a newly developed and proprietary reagent for the transfection of nucleic acids into eukaryotic cells.

LipoGene™ 2000 Plus Transfection Reagent has the highest transfection efficiency in many cell types and formats. And has high transfection efficiency, good repeatability, simple operation, no obvious cytotoxicity features.

LipoGene™ 2000 Plus Transfection Reagent applicable to the plasmid transfection reagent, siRNA, such as a single component of cell transfection, also suitable for multiple plasmid or plasmid transfection and siRNA combination.

After transfection, usually 24 to 48 hours to reach a higher level of protein expression, and the quantity of protein expression in 48 hours is significantly higher than 24 hours.

LipoGene™ 2000 Plus Transfection Reagent can be directly added to cells in culture medium (with or without serum). It is not necessary to remove DNA-Lip2000™ complexes or change medium following transfection. The complexes can be removed after 4-6 hours by replacing with refresh medium.

## Assay Protocols

**1. DNA Transfection**

The following steps apply to 24 plate cultured for mammalian cells. To transfect cells in different tissue culture formats, vary the amounts of LipoGene™ 2000, DNA, cells, and medium used in proportion to the difference in surface area (see table below).The ratio of DNA (in µg):LipoGene™ 2000 (in µl) to use when preparing complexes should be 1:2 to 1:3 for most cell lines. To obtain the highest transfection efficiency and low non-specific effects, Optimizing transfection is possible.

1.1 For adherent cells: One day before transfection, plate cells in growth medium (without antibiotics) so that they will be 90-95% confluent at the time of transfection (0.5-2 x 10 5 cells/well for a 24-well plate).

For suspension cells: On the day of transfection just prior to preparing complexes，plate 4-8 x 10 5 cells/500 µl of growth medium (without antibiotics) in a 24-well plate.

1.2 For each transfection sample, prepare DNA-LipoGene™ 2000 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 LipoGene™ 2000 gently before use, then dilute the appropriate amount in 50 µl of Opti-MEM I Medium (or other medium without serum). Mix gently and incubate for 5 minutes at room temperature.

Note: Combine the diluted LipoGene™ 2000 with the diluted DNA within 30minutes. Longer incubation times may decrease activity. If DMEM is used as a diluent for the LipoGene™ 2000, mix with the diluted DNA within 5 minutes.

c. After the 5 minute incubation, combine the diluted DNA with the diluted LipoGene™ 2000 (total volume is 100 µl). Mix gently and incubate for 20 minutes at room temperature to

allow the DNA-LipoGene™ 2000 complexes to form. The solution may appear cloudy, but this will not inhibit the transfection.

Note: DNA-LipoGene™2000 complexes are stable for at least 5 hours at room temperature.

1.3 Add the 100 µl of DNA-Lip2000™ complexes to each well. Mix gently by rocking the plate back and forth.

1.4 Incubate the cells at 37℃ in a CO 2 incubator for 24-48 hours until they are ready to assay for transgene expression. It is not necessary to remove the complexes or change the medium; However, growth medium may be replaced after 4-6h without loss of transfection activity.

## Optimizing DNA transfection

To obtain the highest transfection efficiency and low non-specific effects, optimize transfection conditions by varying DNA and Lipo™ 2000 concentrations, and cell number. Make sure that cells are greater than 90% confluent and vary DNA (µg) : LipoGene™2000 (µl) ratios from 1:0.5 to 1:5.

## 2. RNAi or siRNA Transfection

The following steps apply to 24 plate cultured for mammalian cells. To transfect cells in different tissue culture formats, vary the amounts of reagents.

2.1 One day before transfection, plate cells in growth medium (without antibiotics) so that they will be 30-50% confluent at the time of transfection.

2.2 For each transfection sample, prepare DNA-LipoGene™ 2000 complexes as follows:

a. Dilute 20 pmol siRNA (The transfection siRNA concentrations for 33 nM) in 50 µl of Opti-MEMI Reduced Serum Medium without serum (or other medium without serum). Mix gently.

b. Mix LipoGene™ 2000 gently before use, then dilute 1 µl LipoGene™ 2000 Plus Transfection Reagent in 50 µl of Opti-MEMI Medium(or other medium without serum). Mix gently and incubate for 5 minutes at room temperature.

Note: Combine the diluted LipoGene™ 2000 with the diluted DNA within 25 minutes.

c. Combine the diluted RNA with the diluted LipoGene™ 2000 (total volume is 100 µl). Mix gently and incubate for 20 minutes at room temperature to allow the DNA-LipoGene™ 2000 complexes to form. The solution may appear cloudy，but this will not inhibit the transfection.

1.3 Add the 100 µl of DNA-LipoGene™ 2000 complexes to each well. Mix gently by rocking the plate back and forth. Incubate the cells at 37℃ in a CO 2 incubator for 24-48 hours until they are ready to assay for transgene expression. Growth medium may be replaced after 4-6 hours without loss of transfection activity.

## Optimizing siRNA Transfection

To obtain the highest transfection efficiency and low non-specific effects, optimize transfection conditions. For 24 wells, siRNA can range from 10 to 15 pmol, LipoGene™ 2000 Plus Transfection Reagent can range from 0.5 to 1.5 µl.

## Scaling Up or Down Transfections

To transfect cells in different tissue culture formats, vary the amounts of LipoGene™ 2000, DNA, cells, and medium used in proportion to the difference in surface area (see table below). With automated, high-throughput systems, larger complexing volumes are recommended for transfections in 96-well plates. Note: You may perform rapid 96-well plate transfections (plate cells and transfect simultaneously) by adding a suspension of cells directly to complexes prepared in the plate. Prepare complexes and 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 DNA-Lip2000™ complexes.

## Note:

1. Using the high purity of DNA or RNA helps to get high transfection efficiency.

2. Before transfection, cells must be in a good state of growth.

3. Preparing Opti-MEM I Medium or other medium without serum by yourself.

4. LipoGene™ 2000 Plus Transfection Reagent can not vortex, mix gently.

5. For your safety and health, please wear a lab coat and a disposable gloves.

| Culture vessel | Vol. of plaing medium | Vol. of dilution medium | DNA transfection | | siRNA transfection | |
|---|---|---|---|---|---|---|
| | | | DNA | Lipo Gene | siRNA | Lipo Gene |
| 96-well | 100 µL | 2×25 µL | 0.2 µg | 0.5 µL | 5 pmoL | 0.25 µL |
| 24-well | 500 µL | 2×50 µL | 0.8 µg | 2.0 µL | 20 pmoL | 1.0 µL |
| 12-well | 1 mL | 2×100 µL | 1.6 µg | 4.0 µL | 40 pmoL | 2.0 µL |
| 6-well | 2 mL | 2×250 µL | 4.0 µg | 10 µL | 100 pmoL | 5.0 µL |
| 60-mm | 5 mL | 2×0.5 mL | 8.0 µg | 20 µL | 200 pmoL | 10 µL |
| 10-cm | 15 mL | 2×1.5 mL | 24 µg | 60 µL | 600 pmoL | 30 µL |