

# Product Information

## LipoGene™ 2000 Plus Transfection Reagent (High efficiency)

Catalog Number: L7003

Product Size: 0.75 mL, 1.5 mL

### Storage

Store at 4°C and protect from light. When stored as directed, product is stable for at least 12 months. **Do not freeze.**

### Description

LipoGene™ 2000 Plus Transfection Reagent is a very efficient and new transfection reagent that achieves the transfection effect of the most mainstream transfection reagent in the world. Suitable for transfection of plasmids, siRNA or other forms of nucleic acids including DNA, RNA, oligonucleotides 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.

The protocol of LipoGene™ 2000 Plus Transfection Reagent is completely the same as Lipofectamine® 2000 Reagent, and the transfection efficiency is equivalent to or slightly higher than Lipofectamine® 2000 Reagent.

LipoGene™ 2000 Plus Transfection Reagent is not only suitable for transfection of single-component such as plasmids and siRNA, 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.

Transfection of siRNA is usually ideal for down-regulation of the target gene after 3-5 days.

LipoGene™ 2000 Plus Transfection Reagent can be directly added to cells in culture medium (with or without serum). However, for the best transfection results, antibiotic-free culture medium is recommended for transfection. 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.

### Protocol

#### 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.

A. For adherent cells: One day before transfection, plate cells in growth medium (without antibiotics) so that they will be 70-90% confluent at the time of transfection ( $0.5-2 \times 10^5$  cells/well for a 24-well plate).

For suspension cells: One day before transfection, plate  $4-8 \times 10^5$  cells/500 µL of growth medium (without antibiotics) in a 24-well plate.

B. For each transfection sample, prepare DNA-LipoGene™



2000 complexes as follows:

- a. Dilute DNA in 50  $\mu\text{L}$  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  $\mu\text{L}$  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 25 minutes.

- c. After the 5 minute incubation, combine the diluted DNA with the diluted LipoGene™ 2000 (total volume is 100  $\mu\text{L}$ ). 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 6 hours at room temperature.

- C. Add the 100  $\mu\text{L}$  of DNA-Lip2000™ complexes to each well. Mix gently by rocking the plate back and forth.
- D. The gene expression is detected after 18-48 h incubation at 37 °C, and the medium is changed after 4-6 h of transfection.

For stable transfection, inoculate fresh culture medium at 1:10 dilution 24 h after transfection, and select medium can be added the next day.

## 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 ( $\mu\text{g}$ ) : LipoGene™ 2000 ( $\mu\text{L}$ ) 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.

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

- B. For each transfection sample, prepare RNA-LipoGene™ 2000 complexes as follows:

- a. Dilute 20 pmol siRNA (The transfection siRNA concentrations for 33 nM) in 50  $\mu\text{L}$  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 1  $\mu\text{L}$  LipoGene™ 2000 Plus Transfection Reagent in 50  $\mu\text{L}$  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 RNA within 25 minutes.

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

C. Add the 100  $\mu\text{L}$  of RNA-LipoGene™ 2000 complexes to each well. Mix gently by rocking the plate back and forth. Incubate the cells at 37°C in a CO<sub>2</sub> incubator for 24-96 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 50 pmol, LipoGene™ 2000 Plus Transfection Reagent can range from 0.5 to 1.5  $\mu\text{L}$ .



## 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.

Culture vessel	Vol. of plating 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

## Notes

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. LipoGene™ 2000 Plus Transfection Reagent should be closed immediately after use to avoid long-term exposure to the air, which will affect the transfection efficiency.
6. For your safety and health, please wear lab coats and disposable gloves.

