PakGent®

# Plasmid Transfection Instruction Manual

## PGbioFect 3000 Transfection Reagent

## Product Description

PGbioFect 3000 is a highly efficient, low-toxicity, and serum-resistant transfection reagent. It features high transfection efficiency, low cytotoxicity, and a simple operation method, making it a widely universal transfection reagent suitable for DNA, RNA, and RNAi transfection, and compatible with hundreds of cell types.

For common cell types, PGbioFect 3000 reagent offers higher efficiency and lower usage compared to other reagents, thus bringing better cost-performance to customers. The 1.5 mL specification product is sufficient to complete up to 1500 transfection reactions (in 24-well plates).

## Shipping and Storage

Shipped with ice packs, stored at 2–8 °C. Do not freeze.

## Ordering Information

| Product Name | Product No. | Specification | Storage |
|---|---|---|---|
| PGbioFect 3000 Transfection Reagent | TR-3000-075 | 0.75 mL | 2–8 °C |
| PGbioFect 3000 Transfection Reagent | TR-3000-150 | 1.5 mL | 2–8 °C |
| PGbioFect 3000 Transfection Reagent | TR-3000-1500 | 15 mL | 2–8 °C |

## Transfection Procedure

**Note 1:** The usage amount of the transfection reagent is affected by cell types and experimental conditions. It is recommended to set gradients for optimization when using it for the first time.

**Note 2:** This product is specially optimized for use in serum-containing and serum-free media. The medium does not need to be changed before transfection; the transfection reagent and sample can be directly mixed and added to the culture medium. For some difficult-to-transfect cells, it is recommended to replace them with serum-free medium before transfection and then switch back to complete medium or add serum after 4–6 hours of transfection.

- Adherent cells: 1 day (20–24 hours) before transfection, digest cells with trypsin and count them. Plate cells (antibiotic-free), and the cell density at the time of transfection should be 70–90%.
- Suspension cells: The cell density at the time of transfection should be 70–90%.

**01. Inoculate cells to 70–90% confluency. Perform transfection according to the following cell counts:**

| Culture Dish Type | 96-Well | 24-Well | 6-Well |
|---|---|---|---|
| Cell Number | 1–4×$10^4$ | 0.5–2×$10^5$ | 0.25–1×$10^6$ |

**02. Take a new EP tube, dilute PGbioFect 3000 transfection reagent with Opti-MEM medium according to the table below, make two replicates, and mix thoroughly.**

| Culture Dish Type | 96-Well | 24-Well | 6-Well |
|---|---|---|---|
| Opti-MEM Medium | 5 µL | 25 µL | 125 µL |
| PGbioFect 3000 | 0.15 µL or 0.3 µL | 0.75 µL or 1.5 µL | 3.75 µL or 7.5 µL |

**03. Take a new EP tube, dilute the DNA sample to be transfected with Opti-MEM medium, prepare the DNA premix, and mix thoroughly.**

| Culture Dish Type | 96-Well | 24-Well | 6-Well |
|---|---|---|---|
| Opti-MEM Medium | 5 µL | 25 µL | 125 µL |
| DNA (0.5–5 µg/µL) | 0.1 µg | 0.5 µg | 2.5 µg |

**04. Take a new EP tube, mix the premixes prepared in steps 2 and 3 at a 1:1 ratio, pipette gently to mix, and let stand at room temperature for 10–15 minutes.**

| Culture Dish Type | 96-Well | 24-Well | 6-Well |
|---|---|---|---|
| Diluted DNA | 5 µL | 25 µL | 125 µL |
| Diluted PGbioFect 3000 | 5 µL | 25 µL | 125 µL |

**05. Add the mixture incubated in the above step to the cells according to the following volumes.**

| Culture Dish Type | 96-Well | 24-Well | 6-Well |
|---|---|---|---|
| DNA-PGbioFect 3000 Complex | 10 µL | 50 µL | 250 µL |
| DNA per Well | 100 ng | 500 ng | 2500 ng |
| PGbioFect 3000 per Well | 0.15 µL or 0.3 µL | 0.75 µL or 1.5 µL | 3.75 µL or 7.5 µL |

**06.** Incubate the transfected cells at 37°C for 2–4 days, and analyze the transfection efficiency and cell status using a microscope.

**Note**: This product is specially optimized. For most cells, medium replacement is not required after transfection. Incubate at 37°C for 2–4 days to detect the gene transfection effect. If required by the experiment, the medium can be replaced at around 4–6 hours after transfection. The incubation time is slightly different and related to cell types.

Appendix: Configuration Table of Common Experimental Systems

| Culture Dish Type | Serum-Free Medium Usage | | DNA Transfection | | siRNA Transfection | |
|---|---|---|---|---|---|---|
| | Cell Culture Medium Volume | Medium Volume for Transfection Reagent Preparatio | DNA (μg) | PGbioFect 3000 Reagent (μL) | siRNA (pmol) | PGbioFect 3000 Reagent (μL) |
| 96-well | 100 μL | 2×5 μL | 0.1 | 0.15, 0.3 | 3 | 0.3 |
| 48-well | 250 μL | 2×12.5 μL | 0.25 | 0.37, 0.75 | 7.5 | 0.75 |
| 24-well | 500 μL | 2×25 μL | 0.5 | 0.75, 1.5 | 15 | 1.5 |
| 12-well | 1 mL | 2×50 μL | 1 | 1.5, 3 | 30 | 3 |
| 6-well | 2 mL | 2×125 μL | 2.5 | 3.75, 7.5 | 75 | 7.5 |
| 60 mm | 5 mL | 2×250 μL | 5.5–11 | 8.25, 16.5 | 166 | 17 |
| 10 cm | 10 mL | 2×500 μL | 14–28 | 21.7, 43.4 | 434 | 43 |
| T75 | 15 mL | 2×750 μL | 20–40 | 29.6, 59.2 | 592 | 59 |
| T175 | 35 mL | 2×1.75 mL | 46–90 | 69, 138 | 1382 | 138 |



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