

## Mergene1000<sup>®</sup> NIH/3T3 Cell-Specific DNA Transfection Reagent

Cat. No.: 164415

Size: 100 $\mu$ L / 0.5mL / 1mL

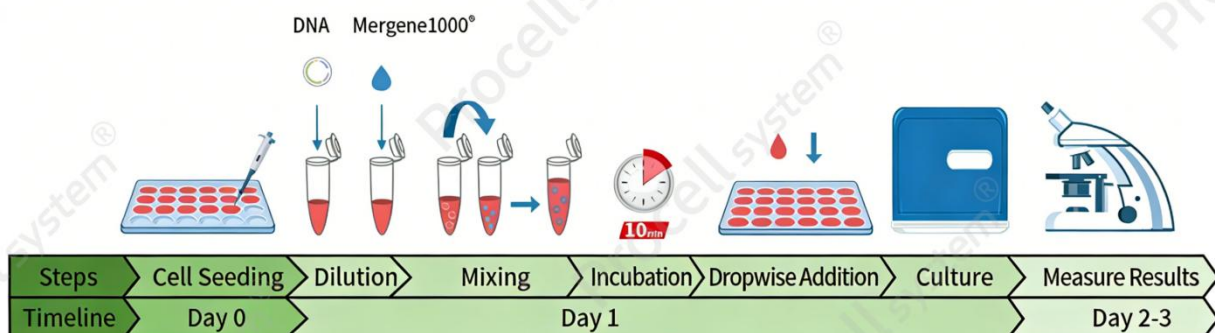
### General Information

Product From	Liquid
Product Color	Colorless transparent
Product Packaging	1 tube
Storage	2-8 $^{\circ}$ C <sup>®</sup>
Expiration Date	18 months
Shipping	Ice bag

### Background

Mergene1000<sup>®</sup> NIH/3T3 Cell-Specific DNA Transfection Reagent is a high-performance DNA<sup>®</sup> transfection reagent designed for the delivery of plasmid DNA. It is characterized by its strong DNA transfection capability and is specifically formulated for use with NIH/3T3 cells, achieving high transfection efficiency. The reagent is distinguished by its low toxicity, excellent stability, ease of operation, and high reproducibility.

### Product Operation Flowchart



### Usage Steps

To transfect NIH/3T3 cells, follow the steps outlined below. Using 24-well plates as an example, mix Mergene1000<sup>®</sup> NIH/3T3 Cell-Specific DNA Transfection Reagent ( $\mu$ L) with plasmid DNA ( $\mu$ g) at a ratio of 2:1. This ratio can be adjusted between 1:1 and 4:1 according to the situation. For other sizes of culture plates or dishes, refer to the recommended transfection amounts provided in Table 1.

#### 1. Cell seeding

The day before transfection, add 500  $\mu$ L DMEM (High glucose) (PM150210) + 10% FBS + 1% P/S (PB180120) medium to each well, inoculate  $0.5 \times 10^5$  cells/well, and culture the cells for 12 hours.

The incubation duration may be adjusted based on the actual conditions of the cells to ensure that the cell confluence reaches 60% to 70% at the time of transfection.

#### 2. Preparation of the transfection complex

- (1) Prepare two sterile centrifuge tubes. In one tube, add 0.5 µg of plasmid and DMEM (High glucose) Medium (PM150210) to a final volume of 10 µL, and gently mix by pipetting. In the other tube, add 1.0 µL of Mergene1000<sup>®</sup> NIH/3T3 Cell-Specific DNA Transfection Reagent and 9.0 µL of DMEM (High glucose) Medium (PM150210) to a final volume of 10 µL and blow and mix.  
**Note:** The above is the amount of preparation for each well of cells. Please calculate the required volumes based on your specific experimental conditions and requirements.
  - (2) Mix the above two equal volume diluents, gently mix by pipetting, and incubate for 10 minutes at room temperature.
3. Cell transfection
- (1) Add the prepared 20 µL transfection complex dropwise to the cells and mixed, incubated at 37°C with 5% CO<sub>2</sub> for culture.
  - (2) After 18-48 hours of incubation, detect gene expression.

**Table 1. Reference dosage of NIH/3T3 cells transfection in different culture vessel**

Culture Vessel	Area	Cell Seeding Density	Inoculation Medium	Diluted Final Volume	Plasmid Transfection	
					Reagent Amount	DNA Amount
96-well	0.3 cm <sup>2</sup>	1-4×10 <sup>4</sup> cells/well	200 µL	2×5 µL	0.4 µL	0.2 µg
24-well	2.0 cm <sup>2</sup>	0.5-1×10 <sup>5</sup> cells/well	500 µL	2×10 µL	1.0 µL	0.5 µg
12-well	4.0 cm <sup>2</sup>	1-2×10 <sup>5</sup> cells/well	1 mL	2×20 µL	2.0 µL	1.0 µg
6-well	10.0 cm <sup>2</sup>	2.5-5×10 <sup>5</sup> cells/well	2 mL	2×50 µL	4.0 µL	2.0 µg
6 cm	20.0 cm <sup>2</sup>	0.5-1×10 <sup>6</sup> cells/well	5 mL	2×0.1 mL	8.0 µL	4.0 µg
10 cm	60.0 cm <sup>2</sup>	1.5-3×10 <sup>6</sup> cells/well	15 mL	2×0.3 mL	24.0 µL	12.0 µg

**Note:** The usage amounts provided in the table are for reference only. The exact amount of DNA used with Mergene1000<sup>®</sup> NIH/3T3 Cell-Specific DNA Transfection Reagent should be optimized according to the cell conditions and other experimental parameters.

## Notes

1. This product is only used for scientific research or further research, not for diagnosis and treatment.
2. The cell inoculation amount and transfection ratio provided above are based on experiments conducted with NIH/3T3 cells and are for reference only. The specific experimental dosage should be adjusted according to the actual conditions.
3. The product is transported with ice bag and can be aliquoted and stored upon use to avoid multiple prolonged openings of the lid.
4. DMEM (High glucose) medium should be prepared separately for the dilution of plasmid DNA and transfection reagents.
5. During transfection, ensure that the degree of cell confluence is not less than 60%, and it is generally maintained at around 60% to 70%. The specific plating density can be adjusted according to the actual conditions of the cells.
6. After transfection, there is no need to remove the transfection complex or replace with fresh culture

medium. The actual operation can be based on the cell status, after transfection culture 4-6 hours to choose to change the medium.

7. The use of high purity endotoxin-free DNA is helpful to obtain higher transfection efficiency.
8. The plasmid concentration and reagent amount should be optimized for the first use to obtain the highest transfection efficiency.
9. For your safety and health, please wear experimental clothes and wear disposable gloves aseptic operation.

### Experimental Results Show (For reference only)

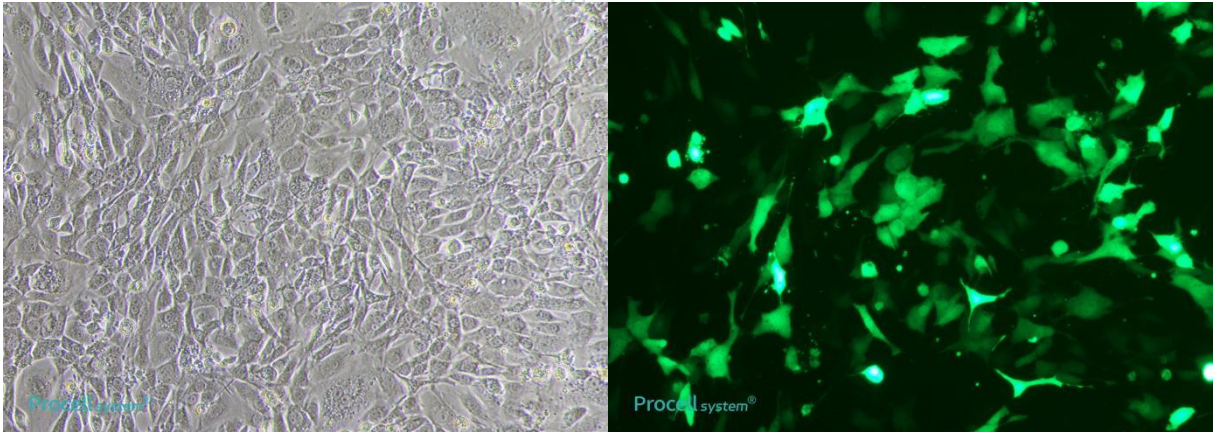


Figure 1. Bright-field and fluorescence images of NIH/3T3 cells transfected with EGFP expression plasmid using Mergene1000<sup>®</sup> NIH/3T3 Cell-Specific DNA Transfection Reagent.

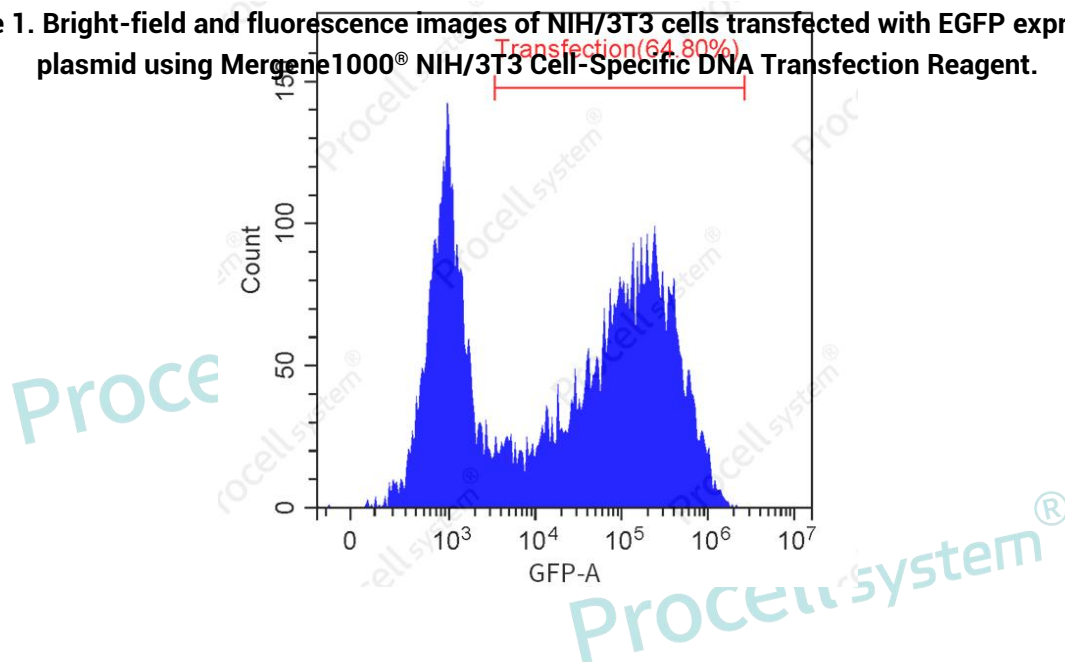


Figure 2. Transfection efficiency of NIH/3T3 cells transfected with EGFP expression plasmid using Mergene1000<sup>®</sup> NIH/3T3 Cell-Specific DNA Transfection Reagent.