

EXOBX® Plasmid DNA Transfection Reagent

Cat. no. R2101

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Cat. No.	Reagent In Kit	Size	Storage
R2101	Plasmid DNA Transfection Reagent	1 mL	4°C

1. Transportation and Storage

- This product is transported at room temperature and should be stored at 4°C. Do not freeze.

2. Important Information (Please read before using the kit)

- 1) To achieve optimal transfection efficiency, plasmid DNA transfection should be performed using cells in the logarithmic growth phase. Typically, transfection is initiated 24-36 hours after cell passage.
- 2) Cell density plays a crucial role in transfection efficiency. It is recommended to select logarithmically growing cells with a confluency of 50-80% for plasmid DNA transfection to ensure high transfection efficiency. Transfection efficiency may be reduced if cell density is either too high or too low.
- 3) The quality of plasmid DNA directly influences transfection efficiency. It is advisable to use high-purity plasmid DNA with an OD260/280 ratio between 1.7 and 1.9 for transfection experiments. The recommended plasmid DNA concentration should exceed 0.2 µg/µL.
- 4) The incubation time after mixing plasmid DNA with R2101 should not be too short. A recommended incubation period of 15-30 minutes (with optimal results at 30 minutes) ensures the formation of transfection complexes, thereby preventing a decrease in transfection efficiency.
- 5) R2101 exhibits minimal cytotoxicity in most cell lines and primary cells. Consequently, the culture medium does not necessarily need to be replaced immediately after transfection; it can be changed after 24 hours if required.
- 6) For suspension cells, it is recommended to replace the culture medium 24 hours after transfection to ensure complete uptake of the transfection complexes by the cells.
- 7) Expression of the transfected plasmid DNA-encoded exogenous gene (e.g., GFP) typically begins 12 hours post-transfection, with 50-70% expression observed at 24 hours and peak expression reached at 48 hours. Replacing the culture medium with fresh medium can further enhance exogenous gene expression. Therefore, to assess gene expression levels, observations and sample collection should be conducted at 48 hours post-transfection to obtain optimal experimental results. Do not freeze R2101, as freezing drastically reduces its efficiency.
- 8) This reagent should be stored at 4°C and must not be frozen. Freezing significantly reduces its transfection efficiency and may even lead to complete loss of activity.
- 9) This product is for research use only!

3. Product Introduction

The EXOBX® Plasmid DNA Transfection Reagent is a non-liposomal DNA transfection reagent specifically designed for eukaryotic cells. It is primarily used for plasmid DNA transfection and exhibits high transfection efficiency with ultra-low cytotoxicity across various cell types. This reagent is widely applied in protein expression, library screening, and lentivirus packaging experiments.

4. Features and Advantages

- 1) High Transfection Efficiency: The transfection reagent efficiently mediates plasmid DNA transfection and facilitates exogenous gene expression across various cell types.
- 2) Low Cytotoxicity: R2101 exhibits minimal cytotoxicity in most cell lines and primary cells. Following transfection, there is no requirement for a medium change.
- 3) Convenient Operation: This reagent is unaffected by antibiotics or serum, eliminating the need for a medium change before transfection.

5. Experimental Procedure

For the transfection experiment, adherent cells cultured in a 6-well plate are used as the experimental system. The procedure is as follows:

- 1) Cell Culture: Cells are collected using trypsin digestion, counted, and seeded into a 6-well plate. After 24 hours, the cell density should reach 50%–80%.
- 2) Preparation of the Transfection Complex: Pipette 200 μL of OPTI-MEM medium (Gibco) into a sterile 1.5 mL EP tube. Sequentially add 2 μg of plasmid DNA (adjust volume based on plasmid concentration) and 6 μL of R2101 transfection reagent. Mix gently 10–20 times and incubate at room temperature for 15–30 minutes (30 minutes is recommended for optimal transfection complex formation).
- 3) Cell Transfection: Add the prepared transfection complex to the cell culture medium, gently swirl to mix, and incubate the cells in a CO_2 incubator for further culture.
- 4) Post-Transfection Handling: Replace the culture medium 12–24 hours after transfection. Assess exogenous protein expression 48 hours post-transfection, for example, by examining GFP fluorescence under a fluorescence microscope.

Table 1. Recommended Amounts of DNA, R2101, and OPTI-MEM

Culture Plate	DNA Amount (μg)	R2101 Volume (μL)	OPTI-MEM Volume (μL)
24-well plate	0.5	1.5	100
6-well plate	2	6	200
Ø 35 mm dish	2	6	200
Ø 60 mm dish	4	12	400
T25 flask	5	15	500

6. Transfection Efficiency Evaluation

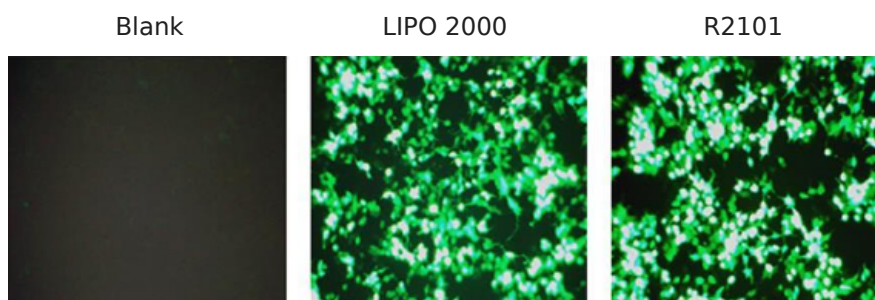


Fig. 1. Green Fluorescent Protein (GFP) Expression Analysis (100× Magnification)

HEK293 cells were seeded in a 6-well plate and transfected with a Green Fluorescent Protein (GFP) plasmid using R2101 and Lipofectamine 2000 (LIPO 2000) after 24 hours. GFP expression was observed and imaged under a fluorescence microscope 48 hours post-transfection.

7. Troubleshooting

Issue	Possible Cause	Solution
Low Transfection Efficiency	The cell line itself has low transfection efficiency.	Increase the amount of plasmid DNA and R2101 used.
	Cells were not in the logarithmic growth phase.	Perform transfection within 24–36 hours after cell passage.
	Cell density was too high or too low.	Adjust the cell seeding density to ensure 50–80% confluency at the time of transfection.
	Low plasmid purity or concentration.	Use plasmid DNA with an OD260/280 ratio of 1.7–1.9 and a concentration of at least 0.2 µg/µL.
	Insufficient plasmid DNA amount.	Refer to the recommended DNA quantities in the manual; ensure that the plasmid DNA amount does not fall below the minimum required per well.
	The target gene was not fully expressed.	Exogenous gene expression begins 24 hours post-transfection and peaks at 48 hours. For optimal detection, assess gene expression at 48 hours post-transfection.
	Insufficient incubation time for transfection complex formation.	After mixing plasmid DNA with R2101, incubate at room temperature for 30 minutes to ensure complete complex formation. Do not disturb the mixture before adding it to the cells.
	Incorrect plasmid DNA to R2101 ratio.	Maintain a plasmid DNA to R2101 ratio of 1 µg:3 µL. Reducing R2101 volume may lower transfection efficiency.

High Cytotoxicity	Specific cell lines are sensitive to R2101.	Reduce the R2101 volume and replace the culture medium 6 hours post-transfection.
	Low cell density.	Increase the cell seeding density to ensure at least 50% confluency before transfection.
	Low plasmid purity.	Use high-purity plasmid DNA prepared with a high-quality plasmid extraction kit.
	Poor cell condition.	Improve cell health or use a different batch of cells.
	Excessive R2101 volume.	Follow the recommended R2101 dosage as specified in the manual.