PT02-ProteanFect™ Max Transfection Kit: Instructions for Use

Product Overview

ProteanFect™ Max Transfection Kit offers a non-viral, non-electroporation, and non-liposomal transfection system utilizing engineered mammalian proteins. This innovative design achieves high transfection efficiency while maintaining a superior safety profile. Specifically developed for hard-to-transfect cell lines and challenging primary cells, the kit ensures robust performance across a broad range of cell types (refer to Table 4). Additionally, it is easily scalable for large-scale experiments and ideal for high-throughput applications.

Component Description

The kit is shipped on dry ice. Once received, store the components as indicated below. The kit includes positive control samples with EGFP-encoding mRNA and plasmid DNA (pDNA) to verify transfection efficiency.

Table 1 Storage Conditions for the Components

Component	Storage
Reagent A	2-8°C
Reagent B	-20°C
Reagent C	2-8°C
EGFP mRNA (1 μg/μL)	-80°C
GFP pDNA (0.5 μg/μL)	-20°C

Note: Avoid repeated freeze-thaw cycles of Reagent B, EGFP mRNA, and EGFP pDNA.

Pre-Experimental Preparation

Cell Condition: Ensure cells are in optimal physiological condition on the day of transfection, with >90% viability. For certain primary cells, such as human primary T cells, proper activation before transfection is crucial for optimal results.

Reagent: Allow Reagents A, B and C to reach room temperature. Briefly mix each reagent by inverting or vortexing prior to use.

Medium: Use Opti-MEM as the recommended medium. Serum-free RPMI 1640 or DMEM can be used as alternatives. Pre-warm the medium to 37°C or room temperature before use.

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Transfection Procedure

Table 2 Transfection Protocol for mRNA per Well of a 96-Well Plate

Steps	Instructions for Cell Lines	Instructions for Primary Cells [a]
1. Transfection Complex Preparation [b]		
1.1 Mix Reagent A with mRNA	Mix 0.5 μg of mRNA with 40 μL of Reagent A.	
	Note: Invert Reagent A briefly before use to ensure uniformity.	
1.2 Add Reagent B	Add 1.4 μL of Reagent B to the mixture. Mix gently by pipetting up and down 20-30 times or vortexing for 10 seconds.	Add 0.7 μL of Reagent B to the mixture. Mix gently by pipetting up and down 20-30 times or vortexing for 10 seconds.
1.3 Add Reagent C	N/A	Add 8 μL of Reagent C to the mixture. Mix gently by pipetting up and down 2-3 times or vortexing for 2-3 seconds.
2. Cell Preparation		
2.1 Suspension cells	Harvest the cells by centrifugation at 300 *g* for 5 minutes. Discard the supernatant and wash cells once with Opti-MEM. Resuspend cells with Opti-MEM and adjust concentration to 5×10^6 - 1×10^7 cells/mL. **Note:** Avoid including FBS in the transfection medium.	
2.2 Adherent cells	Maintain 50%-80% cell confluence. Remove medium, wash cells once with Opti-MEM, then add 20 μL of Opti-MEM. **Note:** Avoid including FBS in the transfection medium. **Optional:** Harvest cells by trypsinization, then resuspend them in Opti-MEM at a concentration of 5×10^6 - 1×10^7 cells/mL for subsequent transfection.	
3. Transfection		
3.1 Mix complex with cells	For suspension cells, mix 40 μL of transfection complex with 20 μL of cell suspension and gently pipet up and down 2-3 times. For adherent cells, apply directly to the cells.	
3.2 Incubation	Incubate the cells with the transfection complex for 45-60 minutes in a cell culture incubator.	Incubate the cells with the transfection complex for 15-30 minutes in a cell culture incubator.
3.3 Termination	Terminate the reaction by adding ≥200 μL of culture medium (10X cell suspension), centrifuge at 300 *g* for 5 minutes, and discard the supernatant. For adherent cells, replace the transfection mixture with ≥200 μL of culture medium (10X cell suspension). **Note:** The cell pellet may adhere to the tube walls. Gently remove the supernatant to minimize cell loss.	
3.4 Post-transfection culture	Incubate transfected cells in culture medium and assess transfection efficiency after 5 to 48 hours, or at an appropriate time.	

FBS, Fetal bovine serum. **a.** Proper activation is crucial for primary cells, such as human primary T cells, which should be stimulated with anti-CD3/CD28 beads or antibodies for 2-10 days to achieve optimal transfection efficiency. **b.** The transfection complex may become slightly viscous during preparation. It can be directly added to cells once prepared without incubation. For optimal results, use the complex within 30 minutes.

Table 3 Transfection Guidelines for Different Culture Formats

Components	Culture Vessels [a]	Cell Lines			Primary Cells	
Reagent A	96-well	40 μL				
	48-well	80 μL				
	24-well	200 μL				
	12-well	600 μL				
	6-well	800 μL				
Nucleic Acids [b]		**DNA**	**mRNA**	**siRNA**	**mRNA**	**siRNA**
	96-well	0.4 μg	0.5 μg	40 pmol	0.5 μg	20 pmol
	48-well	0.8 μg	1 μg	80 pmol	1 μg	40 pmol
	24-well	2 μg	2.5 μg	200 pmol	2.5 μg	100 pmol
	12-well	6 μg	7.5 μg	600 pmol	7.5 μg	300 pmol
	6-well	8 μg	10 μg	800 pmol	10 μg	400 pmol
Reagent B	96-well	1 μL	1.4 μL	1.4 μL	0.7 μL	
	48-well	2 μL	2.8 μL	2.8 μL	1.4 μL	
	24-well	5 μL	7 μL	7 μL	3.5 μL	
	12-well	15 μL	21 μL	21 μL	10.5 μL	
	6-well	20 μL	28 μL	28 μL	14 μL	
Reagent C	96-well	NA			8 μL	
	48-well				16 μL	
	24-well				40 μL	
	12-well				120 μL	
	6-well				160 μL	
Recommended Cell Number (Opti-MEM) [c]	96-well	1×10^5 ~2×10^5 (20 μL)				
	48-well	2×10^5 ~4×10^5 (40 μL)				
	24-well	5×10^5 ~1×10^6 (100 μL)				
	12-well	1.5×10^6 ~3×10^6 (300 μL)				
	6-well	2×10^6 ~4×10^6 (400 μL)				

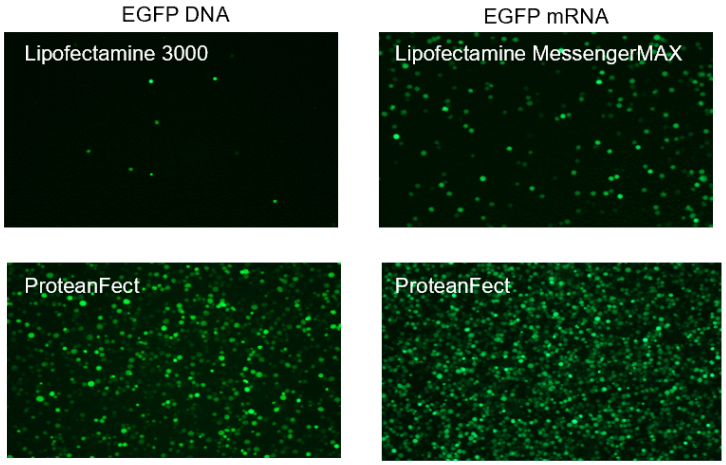
a. For large-scale transfections, such as in 48-well plates or larger formats, it is recommended to use centrifuge tubes for the transfection process. **b.** When co-transfecting multiple nucleic acids, please ensure the total amount of nucleic acids added matches the recommended quantities for each plate format, as outlined in Table 3. **c.** The recommended cell number is primarily for suspension cells. For adherent cells, please adjust the cell number based on confluency.

Table 4 Primary Cells and Cell Lines Successfully Transfected Using ProteanFect™ Max Transfection Kit

Cell Lines		Tested Nucleic Acid Types for Transfection
Primary cells	Human primary T cells	mRNA, siRNA
	Human primary NK cells	mRNA, siRNA
	Human primary monocytes	mRNA, siRNA
	Human primary $CD34^+$ hematopoietic stem cells	mRNA, siRNA
	Mouse primary cardiomyocytes	mRNA, siRNA
	Mouse primary neurons	pDNA, mRNA, siRNA
	Mouse primary glial cells	pDNA, mRNA, siRNA
	Mouse primary T cells	mRNA, siRNA
	Mouse primary NK cells	mRNA, siRNA
	Large yellow croaker primary mesenchymal stem cells	mRNA, siRNA
Cell lines	Jurkat T (human T lymphoblastic leukemia cells)	pDNA, mRNA, siRNA
	LX-2 (human hepatic stellate cells)	pDNA, mRNA, siRNA
	HepG2 (human liver tumor cells)	pDNA, mRNA, siRNA
	THP-1 (human acute monocytic leukemia cell line)	mRNA, siRNA
	Raji (human Burkitt's lymphoma cells)	mRNA, siRNA
	K562 (human chronic myeloid leukemia cells)	pDNA, mRNA, siRNA
	MOLT-16 (human T lymphoblastic leukemia cells)	mRNA, siRNA
	SH-SY5Y (human neuroblastoma cells)	pDNA, mRNA, siRNA
	U2OS (human osteosarcoma cells)	pDNA, mRNA, siRNA
	U937 (human lymphoma cell line)	mRNA, siRNA
	HFF (human foreskin fibroblasts)	pDNA, mRNA, siRNA
	HEK-293 (human embryonic kidney cell line)	pDNA, mRNA, siRNA
	MC38 (mouse colon cancer cells)	mRNA, siRNA
	RAW264.7 (mouse mononuclear macrophage leukemia cells)	mRNA, siRNA
	LLC (mouse Lewis lung cancer cells)	pDNA, mRNA, siRNA
	C2C12 (mouse myoblasts)	pDNA, mRNA, siRNA
	COS7 (African green monkey kidney fibroblast-like cells)	pDNA, mRNA, siRNA

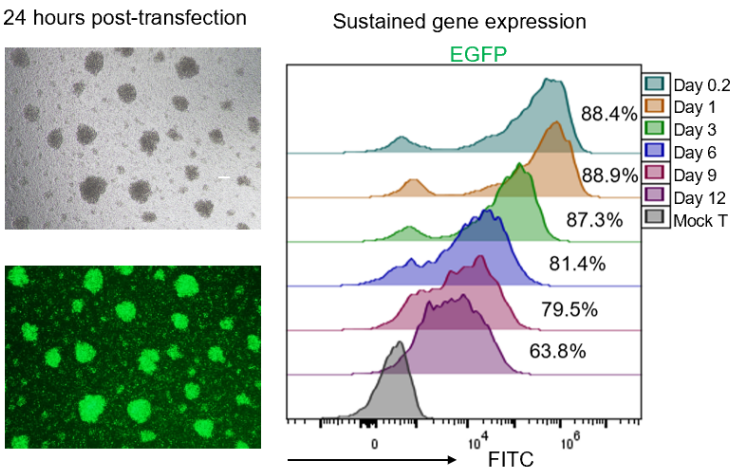
Supporting Data

Case 1: Successful Transfection of Jurkat T Cells



- **Cell Type:** Jurkat T Cells
- **Transfected Nucleic Acid Type:** EGFP pDNA or EGFP mRNA
- **Detection Time:** 48 hours post-pDNA transfection; 24 hours post-mRNA transfection

Case 2: Successful Transfection of primary human T Cells



- **Cell Type:** Primary human T cells continuously activated for 6 days using Dynabeads™ Human T-Expander CD3/CD28 (Thermo Fisher, 11141D).
- **Transfected Nucleic Acid Type:** EGFP mRNA.
- **Detection Time:** Continuous monitoring from 5 hours to 12 days post-transfection.

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Frequently Asked Questions (FAQs) and Troubleshooting Guide

1. Low Transfection Efficiency

1.1 Optimize Transfection Parameters

Optimize transfection parameters for each cell type. **Extended incubation time**: Adjust the incubation time of the transfection complex with cells. The maximum incubation time is 2 hours for cell lines, and 30 minutes for primary cells. **Increase ProteanFect transfection complex**: Consider increasing the amount of transfection complex to improve transfection efficiency.

1.2 Severe Cytotoxicity Caused by Plasmid DNA

The transfection of pDNA into primary cells, such as primary T cells, can induce cytotoxicity and inflammatory responses. Due to the risk of significant toxicity, pDNA transfection is generally not recommended for primary T cells.

1.3 Improve Plasmid DNA Quality

Transfection efficiency is highly dependent on the quality of the plasmid DNA. **Endotoxin-free**: Use an endotoxin-free kit to prepare plasmid DNA. **Optimal concentration**: Ensure an OD260/280 ratio between 1.7 and 1.9, and dilute DNA to 0.5–2 μg/μL using nuclease-free water.

1.4 Improve Cell Condition

For cell lines, transfect cells with >90% viability, confirmed by trypan blue exclusion. Avoid using cells beyond 15 passages, and allow 2-3 passages for recently thawed cells to stabilize before transfection.

For primary cells, proper activation is crucial for optimal transfection efficiency. For example, human primary T cells generally achieve the best transfection results after stimulation with anti-CD3/CD28 activation beads or antibodies for 2-10 days, with peak efficiency typically observed around days 4-6.

1.5 Use Positive Control

We recommend using a 96-well plate format to optimize transfection conditions for a specific cell type, with EGFP mRNA as the positive control.

2. Low Cell Viability

Transfected cells may exhibit transient changes in behavior, but typically, viability will

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be restored by the second day post-transfection.

3. Lack of Pellet Post-Centrifugation

In 96-well formats, it is common for the pellet to be less distinct and may adhere to the tube walls. Gently pipetting can help minimize cell loss.

Contact Information: For further questions, please contact us at: proteanfect@nanoportalbio.com.