ProteanFect™ Max Transfection Protocol for Human Primary T Cells

Genetic engineering enables T cells to effectively treat a variety of diseases, including cancer and autoimmune disorders. However, transfecting T cells often presents significant challenges, especially in complex research involving multiple genes and targets. The ProteanFect™ Max Transfection Kit offers a non-viral, non-electroporation, and non-liposomal transfection system utilizing engineered mammalian proteins. It can efficiently, safely, and conveniently deliver genes into human primary T cells, making it particularly suitable for the delivery of large and multiple gene fragments. This protocol provides a detailed introduction on how to use ProteanFect Max to achieve efficient transfection of human primary T cells.

1. Pre-Experimental Preparation

For human primary T cells, proper culture conditions and activation are crucial for successful transfection.

1.1 Isolation and Activation of Human Primary T Cells

Isolation of Human Primary T Cells: Start with peripheral blood mononuclear cells (PBMCs) from whole blood or the buffy coat layer. T cell subsets (including $CD3^+$ T cells, $CD4^+$ T cells, or $CD8^+$ T cells) can be isolated using commercial negative or positive selection methods.

Activation of Human Primary T Cells: CD3/CD28 activation beads are a common T cell activator, providing the primary and co-stimulatory signals required for T cell activation and proliferation, significantly improving the activation and expansion efficiency of T cells.

1.2 Culture and Passaging of Human Primary T Cells

a) Culture of T Cells: During T cell culture and activation, it is recommended to add recombinant human IL-2 to the culture medium to stimulate the expansion of the T cell population. The concentration of IL-2 should be 300 IU/mL. There is no need to remove the activation beads during the culture process before transfection.

b) Passaging of T Cells: T cells should be passaged every 2 days, maintaining a cell density of 1 × 106 cells/mL.

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c) Timing of Transfection: Proper activation is crucial for human primary T cells. To achieve optimal transfection efficiency, T cells should be stimulated with anti-CD3/CD28 beads or antibodies for 2-10 days. Transfecting cells 4-7 days after activation results in optimal cell viability and transfection efficiency. If the time for cell activation and passaging is too long, the viability and efficiency of the cells after transfection may decrease.

2. Transfection of Human Primary T Cells Using ProteanFect Max

a) **Suitable Nucleic Acids for Transfection:** mRNA and siRNA are suitable for transfection. However, double-stranded DNA transfection may induce cytotoxicity, making plasmid DNA unsuitable for transfecting primary T cells.

b) **Medium for Transfection:** 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.

c) **Preparation of the ProteanFect Max Transfection Complex:** See Tables 1-2 for details. Given that T cells are relatively sensitive to the environment, it is recommended to process the cells after the transfection complex has been prepared. Note that the transfection complex may become slightly viscous during preparation. If it cannot be used within 30 minutes, it is recommended to place it on ice.

d) **Cell Preparation:** Ensure cells are in optimal physiological condition on the day of transfection, with >90% viability. When adjusting the cell density for transfection, avoid repeated centrifugation, as this may increase handling time and potentially affect cell viability and transfection efficiency.

e) **Cell Transfection:** The recommended incubation time is 15-30 minutes. Extending the incubation time may affect cell viability.

f) **Detection of Transfection Efficiency and Cell Viability:** When using a positive control mRNA, EGFP expression can be observed within 5-48 hours post-transfection. Cell viability can be assessed through microscopic observation; viable cells typically grow in clusters. Additionally, cell viability can be further evaluated using methods such as trypan blue staining or flow cytometry.

Table 1 Transfection Protocol for mRNA per Well of a 96-Well Plate

Steps	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 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	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 Human Primary T Cells (Avoid including FBS in the transfection medium)	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.
3. Transfection	
3.1 Mix complex with cells	mix 40 μL of transfection complex with 20 μL of cell suspension and gently pipet up and down 2-3 times.
3.2 Incubation	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. **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 2 Transfection Guidelines for Different Culture Formats

Components	Culture Vessels	Human Primary T 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]		**mRNA**	**siRNA**
	96-well	0.5 µg	20 pmol
	48-well	1 µg	40 pmol
	24-well	2.5 µg	100 pmol
	12-well	7.5 µg	300 pmol
	6-well	10 µg	400 pmol
Reagent B	96-well	0.7 µL	
	48-well	1.4 µL	
	24-well	3.5 µL	
	12-well	10.5 µL	
	6-well	14 µL	
Reagent C	96-well	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 2. **c.** The recommended cell number is primarily for suspension cells. For adherent cells, please adjust the cell number based on confluency.

Example of Transfecting EGFP mRNA into Human Primary T Cells Using ProteanFect Max

Note: The brand and catalog numbers cited in this protocol are provided for reference purposes only. Users are advised to select alternative products based on their specific experimental requirements.

1. Pre-Experimental Preparation

Table 3 The components of the T cell culture medium

Component	Brand and catalog numbers
X-VIVO™ 15 medium	Lonza, 04-418Q
FBS, 10%	Gibco™, 10099141C
Recombinant Human Interleukin-2, 300 IU/mL	/
Penicillin-Streptomycin-Neomycin (PSN) Antibiotic Mixture (Optional)	Gibco™, 15640055

1) Isolation and Activation of Human Primary T Cells

The cell isolation and activation kit utilized in this experiment is the Dynabeads™ Human T-Expander CD3/CD28 (Thermo Fisher, Catalog Number 11141D). For detailed experimental procedures, refer to the official instruction manual.

2) Culture and Passaging

Transfection may be carried out 2-10 days subsequent to the activation of T cells. The prime interval for transfection lies between 4-7 days after activation, a period during which the cells exhibit peak viability and transfection efficiency. Within the framework of this particular experiment, the transfection procedure was initiated 6 days after the activation of the beads.

2. Cell Transfection

The detailed procedure for transfecting the positive control EGFP mRNA using ProteanFect Max is outlined in Table 1

3. Analysis of Cell Viability and Transfection Efficiency

After transfecting T cells with EGFP mRNA, the viability and transfection efficiency can be assessed using fluorescence microscopy and flow cytometry. First, qualitatively assess the EGFP protein expression, cell morphology, and viability of the transfected T cells under a fluorescence microscope (Figure 1). Subsequently, collect the transfected cells for flow cytometry analysis to quantitatively determine the transfection efficiency. After collecting the cells, centrifuge at 300 × g for 5 minutes to remove the culture medium, and resuspend the cell pellet in 1 × DPBS for flow cytometry analysis (Figure 2).

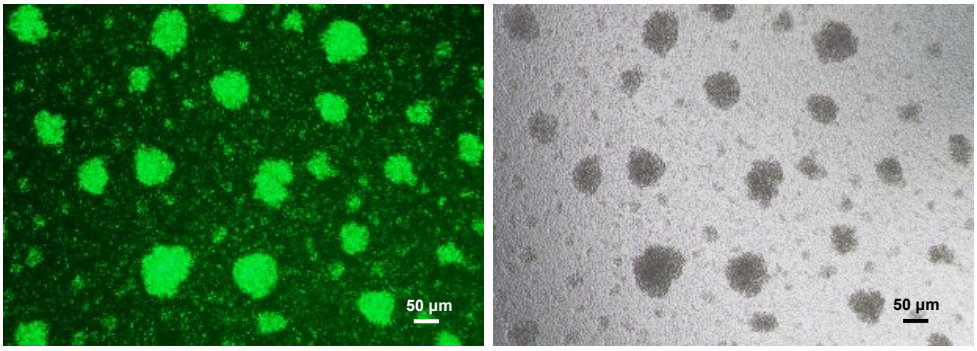

Figure 1. EGFP Expression in Human Primary T Cells Transfected with ProteanFect Max. Fluorescence image (left) and bright-field image (right) demonstrate that one day after transfection with ProteanFect, the cells grow in clusters, indicating high cell viability and unaffected proliferation post-transfection. The majority of cells express green fluorescent protein, highlighting the high transfection efficiency of ProteanFect in human primary T cells.

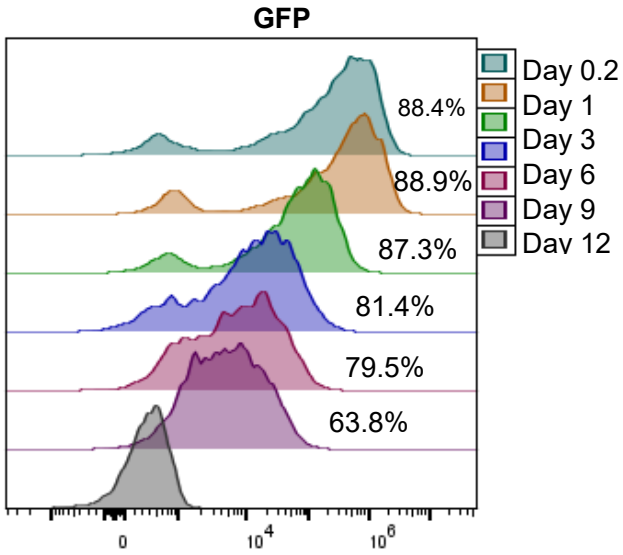

Figure 2. Flow Cytometry Analysis of EGFP Expression in Human Primary T Cells Transfected with ProteanFect Max. Flow cytometry analysis reveals robust EGFP expression in human primary T cells transfected with EGFP mRNA using ProteanFect reagent. Significant EGFP expression is detected as early as 5 hours post-transfection, with sustained green fluorescent protein expression lasting for at least 12 days.

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 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 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.