ProteanFect™ Max Transfection Protocol for Human Primary T Cells

Genetic engineering empowers T cells to effectively treat a variety of diseases, including cancer and autoimmune disorders. However, transfecting T cells often poses significant technical challenges, particularly in complex research scenarios involving multiple genes and targets. The ProteanFect™ Max Transfection Kit provides a non-viral, non-electroporation, and non-liposomal transfection system based on mammalian endogenous proteins. It enables efficient, safe, and convenient gene delivery into human primary T cells, making it especially suitable for the delivery of large and multiple gene fragments. This protocol offers a detailed guide on utilizing ProteanFect™ Max Transfection Kit (PT02) to achieve highly efficient transfection in human primary T cells.

1. Preparation

For human primary T cells, appropriate culture conditions and activation are critical for successful transfection.

1.1 Isolation and Activation of Human Primary T Cells

Isolation of Human Primary T Cells: Begin with peripheral blood mononuclear cells (PBMCs) sourced from whole blood or the buffy coat layer. T cell subsets, such as $CD3^+$ T cells, $CD4^+$ T cells, or $CD8^+$ T cells, can be isolated using commercially available negative or positive selection techniques.

Activation of Human Primary T Cells: CD3/CD28 activation beads are commonly utilized as T cell activators. They supply the primary and co-stimulatory signals essential for T cell activation and proliferation, thereby significantly enhancing the activation and expansion efficiency of T cells.

1.2 Culture and Passaging of Human Primary T Cells

a) Culture of T Cells: During the culture and activation of T cells, it is advisable to supplement the culture medium with recombinant human IL-2 to promote the expansion of the T cell population. The recommended concentration of IL-2 is 300 IU/mL. There is no need to remove the activation beads from the culture medium prior to transfection.

b) Passaging of T Cells: T cells should be passaged every 2 days, maintaining a cell density of 1×10^6 cells/mL.

c) Timing of Transfection: Proper activation is vital for human primary T cells. To achieve optimal transfection efficiency, T cells should be stimulated with anti-CD3/CD28 beads or antibodies for a duration of 2 to 10 days. Prolonged cell activation and passaging may compromise the viability and efficiency of the cells post-transfection.

2. Transfection of Human Primary T Cells Using ProteanFect™ Max Transfection Kit

a) **Suitable Nucleic Acids for Transfection:** mRNA, siRNA and RNP 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:** Opti-MEM is the recommended medium for transfection. Serum-free RPMI 1640 or DMEM can serve as alternatives. Pre-warm the medium to 37°C or room temperature before use.

c) **Preparation of the ProteanFect Max Transfection Complex:** Refer to Tables 1-2 for detailed preparation steps. Given the

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environmental sensitivity of T cells, it is advisable to process the cells after preparing the transfection complex. Note that the transfection complex may become slightly viscous during preparation. If it cannot be used within 30 minutes, place it on ice.

d) **Cell Preparation:** Ensure that cells are in optimal physiological condition on the day of transfection, with a viability of over 90%. 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 to 30 minutes. Prolonging the incubation time may adversely affect cell viability.

f) **Detection of Transfection Efficiency and Cell Viability:** When using a positive control mRNA, EGFP expression can be observed within 5 to 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 (PT02) with mRNA	Mix 0.5 μg of mRNA with 40 μL of Reagent A (PT02). **Note:** Invert Reagent A briefly before use to ensure uniformity.
1.2 Add Reagent B (PT02)	Add 0.7 μL Reagent B (PT02) to the mixture. Mix thoroughly by pipetting 20–30 times or vortexing for 10 s. **Note:** Thorough mixing is essential for optimal performance.
1.3 Add Reagent C (PT02)	Add 8 μL of Reagent C (PT02) 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:** Ensure the transfection medium contains no FBS or serum.
3. Transfection	
3.1 Mix transfection complex with cells	Mix transfection complex with 20 μL of cell suspension in an Eppendorf tube 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	Add ≥200 μL of complete culture medium (at least 10×cell suspension), then transfer the cells from the tube to the culture plate (a well of 96-well plate).
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 point for your experiment.

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 [a]	Human Primary T Cells	
Reagent A (PT02)	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	0.2-1.0 μg	20-40 pmol
	48-well	0.4-2.0 μg	40-80 pmol
	24-well	1.0-5.0 μg	100-200 pmol
	12-well	3.0-15.0 μg	300-600 pmol
	6-well	4.0-20.0 μg	400-800 pmol
Reagent B (PT02)	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 (PT02)	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.**

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Example of Transfecting EGFP mRNA into Human Primary T Cells

Note: The brand and catalog numbers mentioned in this protocol serve as references. Select alternative products based on specific experimental requirements.

1. Preparation

Table 3 The components of 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 used 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 performed 2-10 days after T cell activation. In this experiment, transfection was initiated 6 days after bead activation.

2. Cell Transfection

The detailed procedure for transfecting positive control EGFP mRNA using ProteanFect™ Max Transfection Kit (PT02) is outlined in Table 1.

3. Analysis of Cell Viability and Transfection Efficiency

After transfecting T cells with EGFP mRNA, assess viability and transfection efficiency using fluorescence microscopy and flow cytometry. Fluorescence Microscopy: Qualitatively evaluate EGFP protein expression, cell morphology, and viability of transfected T cells (Figure 1). Flow Cytometry: Collect transfected cells, centrifuge at 300 × g for 5 minutes to remove culture medium, and resuspend the pellet in 1 × DPBS for analysis (Figure 2).

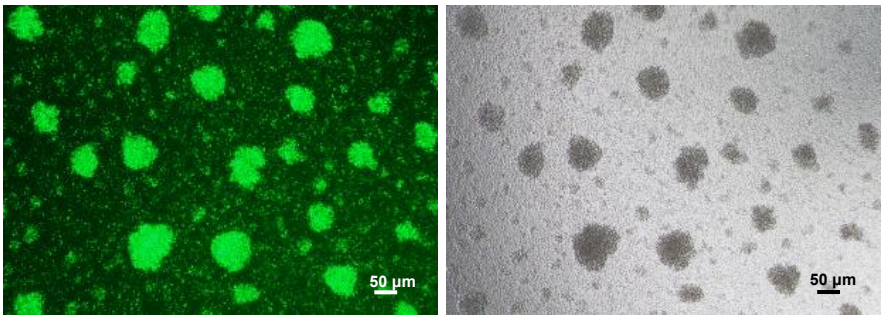

Figure 1. EGFP expression in human primary T cells transfected with ProteanFect™ Max Transfection Kit (PT02). Fluorescence (left) and bright-field (right) images show cell clustering one day post-transfection, indicating high viability and

unaffected proliferation. Most cells express green fluorescent protein, highlighting high transfection efficiency.

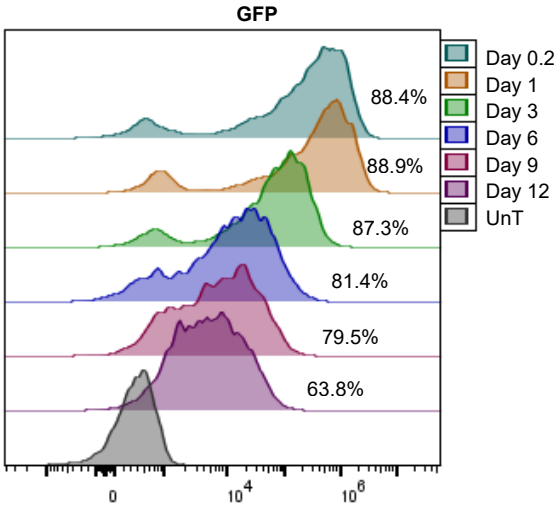

Figure 2. Flow cytometry analysis of EGFP expression in human primary T cells transfected with ProteanFect™ Max Transfection Kit (PT02). Flow cytometry reveals strong EGFP expression in cells transfected with EGFP mRNA. Significant expression is detected as early as 5 hours post-transfection and lasts 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.

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