ProteanFect™ Max Mouse Immunocyte Transfection Protocol for Mouse Primary T Cells

As immunological research advances, there is an increasing demand for studies involving animals with fully functional immune systems, particularly in the fields of CAR-T cell therapy and autoimmune disease research. However, transfecting mouse primary T cells remains challenging, especially in experiments involving multiple genes and targets. The ProteanFect™ 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. The ProteanFect™ Max Mouse Immunocyte Transfection Kit is specifically designed for efficient delivery, particularly suitable for multiple gene fragments into primary mouse immune cells. This protocol provides a detailed guide on using the PT03 kit to achieve efficient transfection in mouse primary T cells.

1. Pre-Experimental Preparation

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

1.1 Isolation and Activation of Mouse Primary T Cells

Isolation of Mouse Primary T Cells: T cells, including CD3+ T cells, CD4+ T cells, CD8+ T cells and T regulatory cells (Tregs), can be isolated from the spleen using negative or positive selection kits.

Activation of Mouse Primary T Cells: CD3/CD28 activation beads provide the primary and co-stimulatory signals required for T cell activation and expansion, thereby significantly enhancing the efficiency of T cell activation and proliferation. Stimulating T cells with CD3/CD28 antibodies mimics the dual-signal action required for T cell activation. This method is also widely used for T cell activation and expansion.

1.2 Culture and Passaging of Mouse 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.

c) Timing of Transfection: To achieve optimal transfection efficiency, T cells should be stimulated with anti-CD3/CD28 beads or antibodies for 2-4 days. 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 Mouse Primary T Cells Using ProteanFect Max Mouse Immunocyte Transfection Kit

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:** If precipitation occurs in Reagent C (for Mouse Immunocyte), heat to 65°C until fully dissolved before use. 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 Mouse Immunocytes [a]
1. Transfection Complex Preparation [b]	
1.1 Mix Reagent A (for Mouse Immunocyte) with mRNA	Mix 0.5 μg of mRNA with 40 μL of Reagent A (for Mouse Immunocyte). **Note:** Invert Reagent A (for Mouse Immunocyte) briefly before use to ensure uniformity.
1.2 Add Reagent B (for Mouse Immunocyte)	Add 0.7 μL of Reagent B (for Mouse Immunocyte) to the mixture. Mix gently by pipetting up and down 20-30 times or vortexing for 10 seconds.
1.3 Add Reagent C (for Mouse Immunocyte)	Add 10 μL of Reagent C (for Mouse Immunocyte) to the mixture. Mix gently by pipetting up and down 2-3 times or vortexing for 2-3 seconds. **Note:** If precipitation occurs in Reagent C (for Mouse Immunocyte), heat to 65°C until fully dissolved before use.
2. Cell Preparation	
2.1 Mouse 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 the cells once with Opti-MEM. Resuspend the cells with Opti-MEM and adjust the cell concentration to 1×10^7 – 1.5×10^7 cells/mL. **Note:** Avoid including FBS in the transfection medium.
3. Transfection	
3.1 Mix transfection 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. 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 the 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 mouse primary T cells, which should be stimulated with anti-CD3/CD28 beads or antibodies 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	Mouse Primary T Cells	
Reagent A (for Mouse Immunocyte)	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 (for Mouse Immunocyte)	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 (for Mouse Immunocyte)	96-well	10 μL	
	48-well	20 μL	
	24-well	50 μL	
	12-well	150 μL	
	6-well	200 μL	
Recommended Cell Number (Opti-MEM) [c]	96-well	2×10^5 ~ 3×10^5 (20 μL)	
	48-well	4×10^5 ~ 6×10^5 (40 μL)	
	24-well	1×10^6 ~ 1.5×10^6 (100 μL)	
	12-well	3×10^6 ~ 4.5×10^6 (300 μL)	
	6-well	4×10^6 ~ 6×10^6 (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 CD3/CD28 antibody-activated mouse primary T cells using ProteanFect Max Mouse Immunocyte Transfection Kit

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

1.1 Isolation and Activation of Mouse Primary T Cells

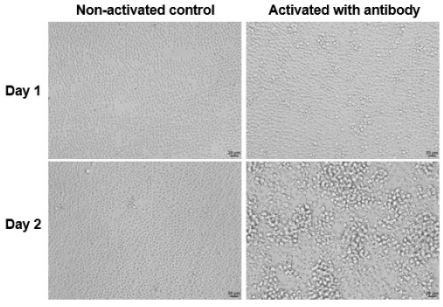
The cell isolation kit used in this experiment is the EasySep™ Mouse T Cell Isolation Kit (Stem Cell, Cat. No. 19851). For detailed experimental procedures, refer to the official instruction manual. The isolated cells were activated using the following antibodies: anti-mouse CD3 (Invivo Mab, Cat. No. BE0002) and anti-mouse CD28 (Invivo Mab, Cat. No. BE0015-1). The specific steps are as follows:

Antibody Coating: In a 24-well plate, wet and wash each well twice with PBS. Add the antibody dilution solution (1:2000, anti-mouse CD3/CD28 antibodies diluted in PBS, resulting in final concentrations of anti-mouse CD3: 4.21 μg/mL and anti-mouse CD28: 4.53 μg/mL). Add 400-500 μL of the dilution solution per well and incubate at 37℃ for 5 hours.

Cell Activation: Suspend the T cells isolated from the mouse spleen in complete culture medium and adjust the cell density to 2 × 10^6 cells/mL. Remove the coating solution from the prepared 24-well plate and add 1 mL of the T cell suspension to each well. Continue culturing to fully activate the T cells.

1.2 Cell Culture

Transfection carried out 2 days subsequent to the activation of T cells. The morphologies of activated and non-activated mouse primary T cells are illustrated in Figure 1.



Email: proteanfect@nanoportalbio.com

Figure 1. Bright-field images of non-activated and antibody-activated mouse primary T cells. Mouse primary T cells activated with antibodies typically appear larger than non-activated cells and begin to form cell clusters after 2 days of activation.

2. Cell Transfection

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

3. Analysis of Transfection Efficiency

At 24 hours post-transfection with EGFP mRNA, cells were collected for flow cytometry to quantitatively assess transfection efficiency. Following cell collection, the culture medium was removed by centrifugation, and the cells were resuspended in 1 × DPBS for flow cytometry analysis (Figure 2).

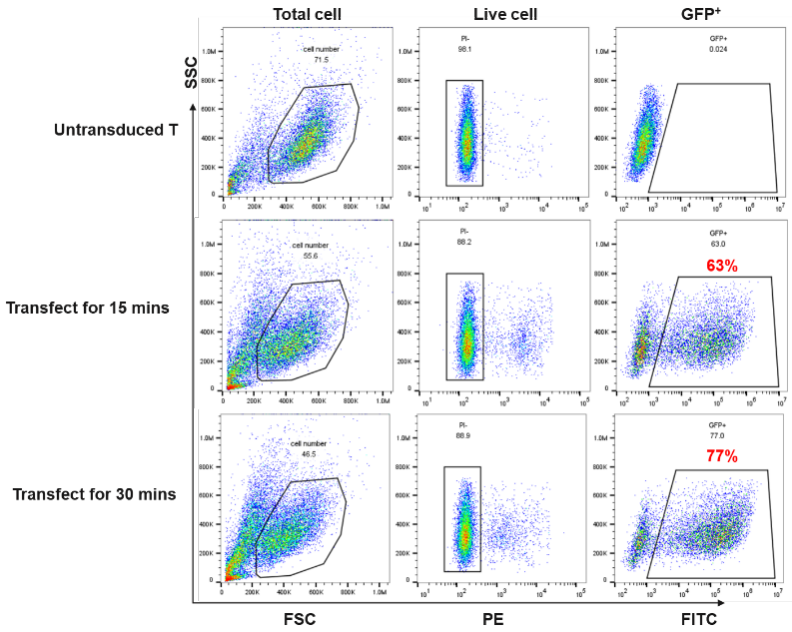


Figure 2. Flow Cytometry Analysis of EGFP Expression in Mouse Primary T Cells (Antibody Activation). Mouse primary T cells were activated with antibodies for 2 days and then transfected for 15 minutes and 30 minutes, respectively. EGFP protein expression rates of 63% and 77% were detected 24 hours post-transfection.

Example of transfecting EGFP mRNA into CD3/CD28 magnetic beads-activated mouse primary T cells using ProteanFect Max Mouse Immunocyte Transfection Kit

1. Pre-Experimental Preparation

The components of the T cell culture medium used in this experiment are listed in Table 3.

1.1 Isolation and Activation of Mouse Primary T Cells

The cell isolation kit used in this experiment is the EasySep™ Mouse T Cell Isolation Kit (Stem Cell, Cat. No. 19851). For detailed experimental procedures, refer to the official instruction manual. The cell activation kit utilized is the Dynabeads™ Mouse T-Expander CD3/CD28 (Thermo Fisher, Catalog Number 11452D). For detailed experimental procedures, refer to the official instruction manual.

1.2 Cell Culture and Passaging

Cells were cultured in the aforementioned medium and continuously activated with magnetic beads until Day 3 for transfection.

2. Cell Transfection

The detailed procedure for transfecting the positive control EGFP mRNA 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 3). 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 4).

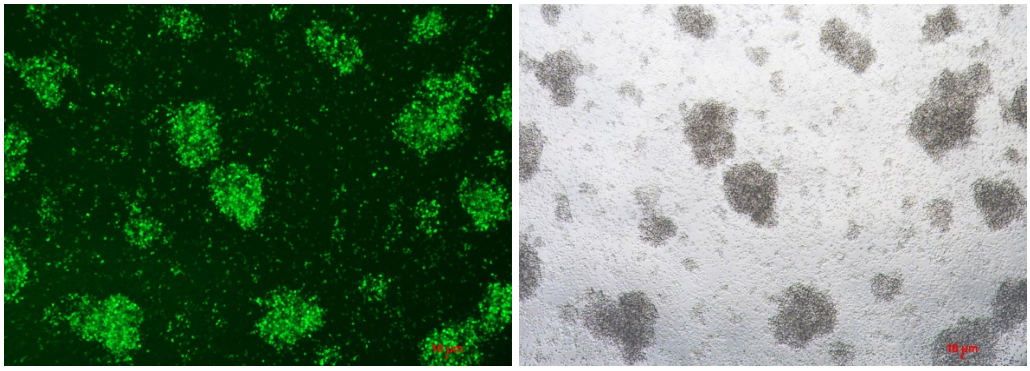


Figure 3. EGFP Expression in Mouse Primary T Cells Transfected with ProteanFect Max Mouse Immunocyte Kit. Fluorescence image (left) and bright-field image (right) demonstrate that one day after transfection with ProteanFect, the cells grow in clusters, indicating high cell viability and unaffected proliferation post-transfection. The majority of cells express green fluorescent protein, highlighting the high transfection efficiency of ProteanFect in human primary T cells.

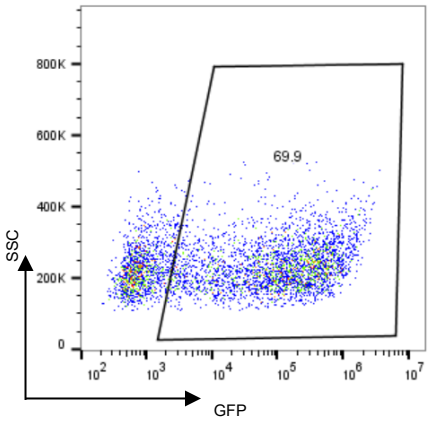


Figure 4. Flow Cytometry Analysis of EGFP Expression in Mouse Primary T Cells Transfected with ProteanFect Max Mouse Immunocyte Kit. Flow cytometry analysis reveals robust EGFP expression in mouse primary T cells transfected with EGFP mRNA.

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 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 Cell Condition

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

1.4 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.