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

As immunological research advances, there is a growing need for animal studies involving fully functional immune systems, particularly in areas such as CAR-T cell therapy and autoimmune disease research. However, transfecting mouse primary T cells remains technically challenging, especially in experiments requiring the delivery of multiple genes or targeting multiple loci. The ProteanFect™ Transfection Kit provides a non-viral, non-electroporation, and non-liposomal transfection system based on engineered mammalian proteins. This innovative design enables high transfection efficiency while ensuring an excellent safety profile. Specifically, the ProteanFect™ Max (PT03) Mouse Immunocyte Transfection Kit is optimized for efficient delivery of multiple gene fragments into primary mouse immune cells. This protocol details a step-by-step procedure for achieving highly efficient transfection of mouse primary T cells using the PT03 kit.

1. Preparation

Successful transfection of mouse primary T cells depends on proper culture conditions and effective activation.

1.1 Isolation and Activation of Mouse Primary T Cells

- **Isolation:** T cell subsets—including $CD3^+$, $CD4^+$, $CD8^+$ T cells, and regulatory T cells (Tregs)—can be isolated from mouse spleen using magnetic bead-based negative or positive selection kits.
- **Activation:** CD3/CD28 activation beads provide both primary and co-stimulatory signals essential for T cell activation and expansion. This approach mimics physiological T cell stimulation and is widely used to enhance activation and proliferation efficiency.

1.2 Culture and Passaging of Mouse Primary T Cells

- **Culture:** During the culture and activation period, recombinant human IL-2 should be supplemented in the medium at a concentration of 300 IU/mL to promote T cell expansion. The CD3/CD28 activation beads do not need to be removed prior to transfection.
- **Passaging:** T cells should be passaged every 2 days to maintain optimal growth and viability.
- **Timing of Transfection:** For best transfection outcomes, perform transfection 2–4 days after stimulation with CD3/CD28 beads or antibodies. Prolonged activation and passaging may reduce post-transfection cell viability and transfection efficiency.

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 (PT03), heat to 65°C until

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fully dissolved before use. Refer to Tables 1-2 for detailed preparation steps. Given the 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 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. 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 5-48 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 (PT03) with mRNA	Mix 0.5 μg of mRNA with 40 μL of Reagent A (PT03). **Note:** Invert Reagent A briefly before use to ensure uniformity.
1.2 Add Reagent B (PT03)	Add 0.7 μL of Reagent B (PT03) to the mixture. Mix thoroughly by pipetting 20 – 30 times or vortexing for 10 s.
1.3 Add Reagent C (PT03)	Add 10 μL of Reagent C (PT03) 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 (PT03), heat to 65°C until fully dissolved before use.
2. Cell Preparation	
2.1 Mouse Primary T Cells	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:** 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 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 (PT03)	96-well	40 μL	
	48-well	80 μL	
	24-well	200 μL	
	12-well	600 μL	
	6-well	800 μL	
Nucleic Acids [b]		**mRNA**	**Nucleic Acids [b]**
	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 (PT03)	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 (PT03)	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.**

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Example of transfecting EGFP mRNA into CD3/CD28 antibody-activated mouse 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

1.1 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, rinse each well twice with PBS. Prepare the antibody dilution solution by diluting anti-mouse CD3/CD28 antibodies in PBS at a ratio of 1:2000, yielding final concentrations of 4.21 μg/mL for anti-mouse CD3 and 4.53 μg/mL for anti-mouse CD28. Add 400–500 μL of the diluted antibody solution per well. Incubate the plate at 37 °C for 5 hours.

Cell Activation: Suspend the isolated mouse splenic T cells in complete culture medium and adjust the cell density to 2×10^6 cells/mL. Remove the coating solution from the pre-coated 24-well plate. Add 1 mL of the T cell suspension into each well. Continue culturing under appropriate conditions to achieve full T cell activation.

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.

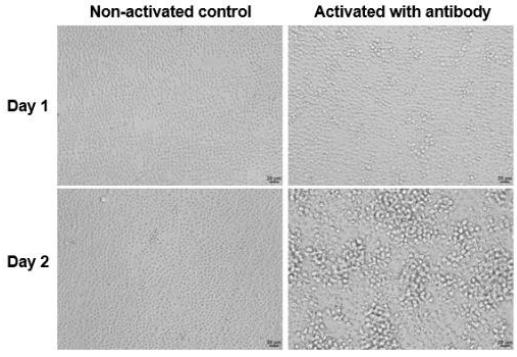

Figure 1. Bright-field images of non-activated and antibody-activated mouse primary T cells. Mouse primary T cells activated with antibodies typically appear larger than non-activated cells and begin to form cell clusters after 2 days of activation primary T cells are illustrated in Figure 1.

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).

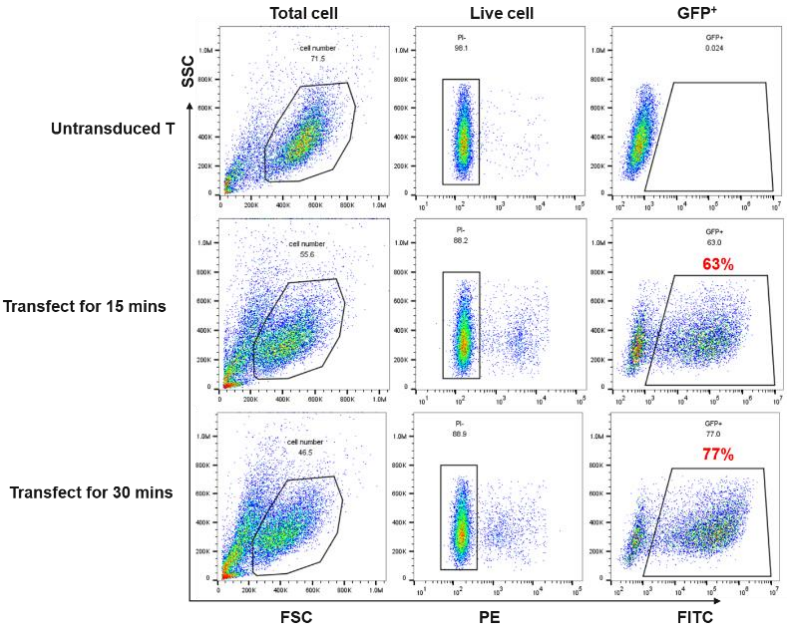

Figure 2. Flow cytometry analysis of EGFP expression in mouse primary T cells (antibody activated). Mouse primary T cells were activated with antibodies for 2 days and then transfected for 15 minutes and 30 minutes, respectively. EGFP protein expression rates of 63% and 77% were detected 24 hours post-transfection.

Example of transfecting EGFP mRNA into CD3/CD28 magnetic beads-activated mouse primary T cells

1. 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). 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

Prior to transfection on **Day 3**, cells were maintained in the described medium under continuous activation with magnetic beads.

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

Following transfection of T cells with EGFP mRNA, viability and transfection efficiency are assessed using fluorescence microscopy and flow cytometry. First, EGFP expression, cell morphology, and viability are qualitatively examined under a fluorescence microscope (Figure 3). For quantitative analysis, the transfected cells are then harvested and prepared for flow cytometry. Briefly, the cells are centrifuged at 300 × g for 5 min, the supernatant is removed, and the pellet is resuspended in 1× DPBS for analysis (Figure 4).

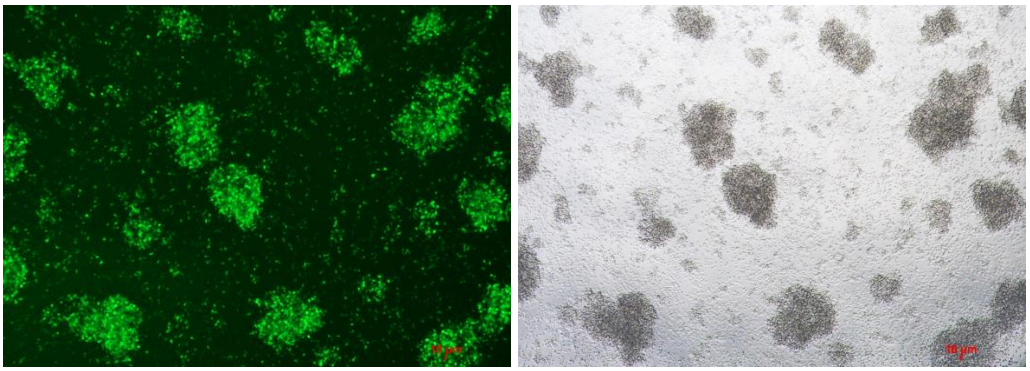

Figure 3. EGFP expression in mouse primary T cells (beads activated) transfected with ProteanFect™ Max Mouse Immunocyte Transfection Kit (PT03). Fluorescence image (left) and bright-field image (right) demonstrate that one day after transfection with ProteanFect, the cells grow in clusters, indicating high cell viability and unaffected proliferation post-transfection. The majority of cells express green fluorescent protein, highlighting the high transfection efficiency of ProteanFect in mouse primary T cells.

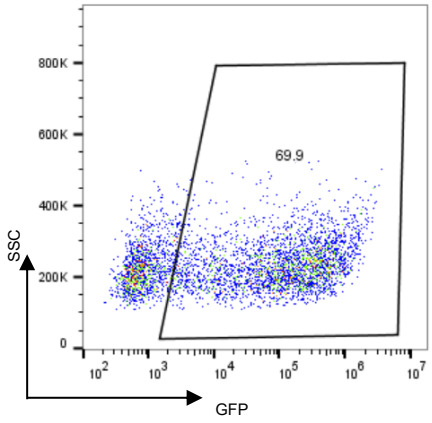

Figure 4. Flow cytometry analysis of EGFP Expression in mouse primary T cells (beads activated) transfected with ProteanFect™ Max Mouse Immunocyte Transfection Kit (PT03). Flow cytometry analysis reveals robust EGFP expression in mouse primary T cells transfected with EGFP mRNA.

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.

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