

Product Overview

ProteanFect™ Max Mouse Immunocyte Transfection Kit offers a non-viral, non-electroporation, and non-liposomal transfection system utilizing engineered mammalian proteins. This innovative system delivers high transfection efficiency while maintaining an excellent safety profile. Specifically designed for primary mouse immune cells, including T cells, NK cells, and $\gamma\delta$ T cells, the kit ensures reliable performance with these sensitive immune cell types.

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 to verify transfection efficiency.

Table 1 Storage Conditions for the Components

Component	Storage
Reagent A (for Mouse Immunocyte)	2-8°C
Reagent B (for Mouse Immunocyte)	-20°C
Reagent C (for Mouse Immunocyte)	2-8°C
EGFP mRNA (1 $\mu\text{g}/\mu\text{L}$)	-80°C

Note: Avoid repeated freeze-thaw cycles of Reagent B (for Mouse Immunocyte) and EGFP mRNA.

Pre-Experimental Preparation

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

Reagent: Allow Reagents A-C (for Mouse Immunocyte) to reach room temperature and briefly mix 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.

PT03-ProteanFect™ Max Mouse Immunocyte Transfection Kit: Instructions for Use

Transfection Procedure

Table 2 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.
1.2 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 Suspension 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: Avoid including FBS in the transfection medium.
2.2 Adherent cells	Maintain 50%-80% cell confluence. Remove medium, wash the cells once with Opti-MEM, and then add 20 µL of Opti-MEM. Note: Avoid including FBS in the transfection medium. Optional: Harvest the cells by trypsinization, then resuspend them in Opti-MEM at a concentration of 1×10 ⁷ – 1.5×10 ⁷ cells/mL for subsequent transfection.
3. Transfection	
3.1 Mix transfection 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 it directly onto the cells.
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.

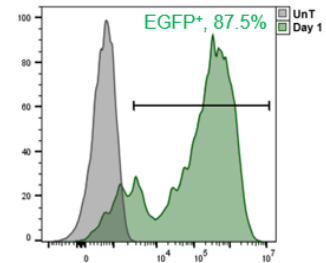
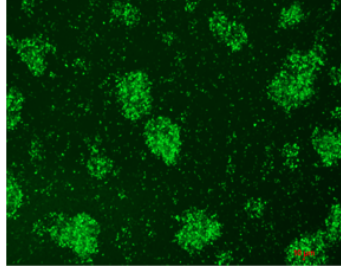
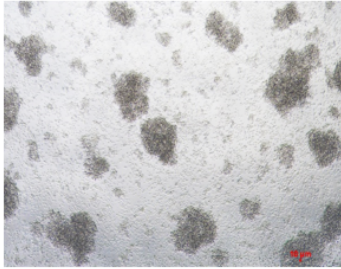
Table 3 Transfection Guidelines for Different Culture Formats

Components	Culture Vessels ^a	Primary Mouse Immunocyte	
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 3. **c.** The recommended cell number is primarily for suspension cells. For adherent cells, please adjust the cell number based on confluency.

Supporting Data

Successful Transfection of Mouse Primary T cells



- **Cell Type:** Mouse primary T cells isolated from spleens. The cells were continuously activated using Dynabeads™ Mouse T-Expander CD3/CD28 (Thermo Fisher, 11452D) for 3 days.
- **Transfected Nucleic Acid Type:** EGFP mRNA.
- **Detection Time:** 24 hours post-mRNA transfection.

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.