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 B cells, the kit ensures reliable performance in 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 (~1000nt) to verify transfection efficiency.

Table 1 Storage Conditions for the Components

Component	Storage
Reagent A (PT03)	2-8℃
Reagent B (PT03)	-20℃
Reagent C (PT03)	2-8℃
EGFP mRNA (1 μg/μL)	-20℃

Note: Avoid freeze–thawing Reagent B more than 10 times, and prepare aliquots of at least 20 μL. Positive controls, given their small volume, do not require this limit and can follow standard handling guidelines for mRNA.

In 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, B and C to reach room temperature. Mix thoroughly by gentle inversion or brief vortexing before use. If precipitation occurs in Reagent C, heat to 65℃ until fully dissolved before use.

Nucleic acids: High-purity nucleic acids are essential for efficient transfection. All nucleic acids should be dissolved in nuclease-free water, and the final concentration adjusted to 0.5– 2 μg/μL before use.

Transfection Medium: Fetal bovine serum (FBS) or other serum markedly impairs transfection, and additional proteins or excess salt ions may also interfere. Use Opti-MEM as the recommended medium; Serum-free RPMI 1640 or DMEM can be used as alternatives. Pre- warm the medium to 37℃ or room temperature before use.

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Transfection Procedure

Important Tip: Thorough mixing during complex preparation is crucial for proper coacervate formation — this is the key difference from liposome-based transfection. Inadequate mixing is a common reason for failed transfections

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 (PT03) with mRNA	Mix 0.2-1.0 μg of mRNA with 40 μL of Reagent A (PT03). Briefly invert the tube before use to ensure reagent uniformity.
1.2 Add Reagent B (PT03)	Add 0.7 μL of Reagent B (PT03) to the mixture. Mix gently by pipetting up and down 20-30 times or vortexing for 10 seconds. **Note:** Thorough mixing is essential for optimal performance.
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 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:** Ensure the transfection medium contains no FBS or serum.
2.2 Adherent cells	Ensure cells are at 50–80% confluency. Remove culture medium, wash once with Opti-MEM, and add 20 μL Opti-MEM. Adherent cells may also be trypsinized and transfected in suspension (follow instructions for suspension cells). **Note:** Suspension transfection may yield higher efficiency for some adherent lines. Consider testing both formats.
3. Transfection	
3.1 Mix transfection complex with cells	For suspension transfection, mix the transfection complex with 20 μL of cell suspension and gently pipet up and down 2-3 times. For adherent transfection, apply directly to the seeded cells.
3.2 Incubation	Incubate the cells with the transfection complex for 15-30 minutes in a cell culture incubator.
3.3 Termination	For suspension transfection, add ≥200 μL of culture medium (10×cell suspension), then transfer the cells from the tube to the culture plate. For adherent transfection, replenish with ≥200 μL of culture medium.
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 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.**

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Table 3 Transfection Guidelines for Different Culture Formats

Components	Culture Vessels [a]	Primary Mouse Immunocyte	
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**	**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 (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	$2\times10^5 \sim 3\times10^5$ (20 µL)	
	48-well	$4\times10^5 \sim 6\times10^5$ (40 µL)	
	24-well	$1\times10^6 \sim 1.5\times10^6$ (100 µL)	
	12-well	$3\times10^6 \sim 4.5\times10^6$ (300 µL)	
	6-well	$4\times10^6 \sim 6\times10^6$ (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.**

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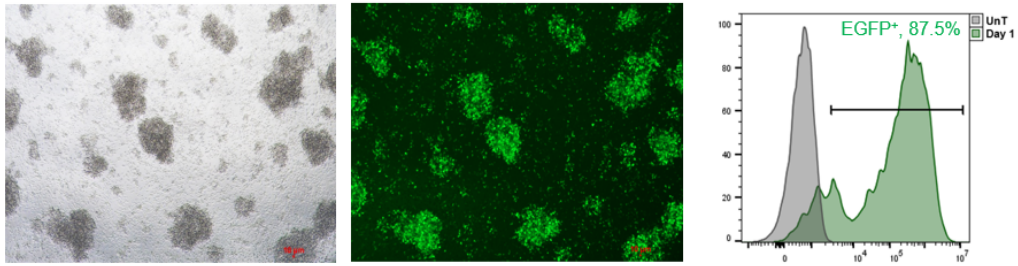
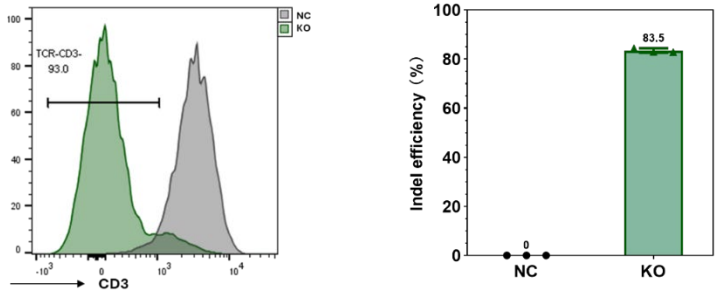
Supporting Data

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.

Successful gene knockout of primary human T cells



- **Cell Type:** Mouse primary T cells
- **Transfection Complex:** Reagent A, B, C with cas9 protein and sgRNA targeting mouse *Trac* gene
- **Detection Time:** 72 hours post-transfection
- **Detection Method**: Flow cytometry analysis of TCR protein expression reveals that approximately 93% of T cells in the positive control group were CD3-TCR complex negative (see the left figure). Sanger sequencing following PCR amplification of the target region (forward primer sequence: CACTGGCATCTGAGTTCTGA, reverse primer sequence: TGTCATGTTCTCTTGTCTGC), analyzed by TIDE, reveals that the average editing ratio in the total cell population was about 83% (see the right figure).

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Transfection Guidelines for CRISPR

(*0.8 μg of Cas9 protein is approximately 5 pmol, and 0.3 μg of sgRNA is approximately 10 pmol.)

Components	Culture Vessels	Mouse Immunocytes	
Reagent A	96-well	40 μL	
	48-well	80 μL	
	24-well	200 μL	
	12-well	600 μL	
	6-well	800 μL	
CRISPR		**Cas9 mRNA/sgRNA**	**Cas9 protein/sgRNA***
	96-well	0.25 μg /0.25 μg	0.8 μg/0.3 μg
	48-well	0.5 μg /0.5 μg	1.6 μg/0.6 μg
	24-well	1.25 μg /1.25 μg	4 μg/1.5 μg
	12-well	3.75 μg /3.75 μg	7.5μg/4.5 μg
	6-well	5 μg /5 μg	16 μg/6 μg
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	10 μL	
	48-well	20 μL	
	24-well	50 μL	
	12-well	150 μL	
	6-well	200 μL	
Recommended Cell Number (Opti-MEM)	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)	

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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.