Product Overview

ProteanFect CRISPRMax Mouse Immunocyte Gene Editing 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 ProteanFectTM CRISPRMax Mouse Immunocyte Gene Editing Kit is specifically designed for effective gene editing by delivering Cas9 mRNA and sgRNA into primary mouse immune cells. It excels in engineering sensitive mouse immune cells, including T cells, NK cells, and γδ T cells.

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 as well as single-guide RNA (sgRNA) targeting the mouse ***Trac*** gene.

Table 1 Storage Conditions for the Components

Component	Storage
Reagent A (for Mouse Immunocyte CRISPRMax)	2-8 ℃
Reagent B (for Mouse Immunocyte CRISPRMax)	-20 ℃
Reagent C (for Mouse Immunocyte CRISPRMax)	2-8 ℃
EGFP mRNA (1 μg/μL)	-80 ℃
Mouse ***Trac***-sgRNA (1 μg / μL)	-80℃

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

The targeting sequence of mouse ***Trac***-sgRNA is TATGGATTCCAAGAGCAATG.

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 CRISPRMax) 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.

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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 CRISPRMax) with mRNA	Mix 0.25 μg Cas9 mRNA and 0.25 μg sgRNA with 40 μL of Reagent A (for Mouse Immunocyte CRISPRMax). **Note:** Invert Reagent A (for Mouse Immunocyte CRISPRMax) briefly before use to ensure uniformity.
1.2 Add Reagent B (for Mouse Immunocyte CRISPRMax)	Add 0.7 μL of Reagent B (for Mouse Immunocyte CRISPRMax) 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 CRISPRMax)	Add 10 μL of Reagent C (for Mouse Immunocyte CRISPRMax) 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 CRISPRMax), 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 1×107 – 1.5×10⁷ 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 1×107 – 1.5×10⁷ 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 evaluate the editing efficiency of the target genes after 48 to 72 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.

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Table 3 Transfection Guidelines for Different Culture Formats

Components	Culture Vessels a	Primary Mouse Immunocyte
Reagent A (for Mouse Immunocyte CRISPRMax)	96-well	40 µL
	48-well	80 µL
	24-well	200 µL
	12-well	600 µL
	6-well	800 µL
Cas9 mRNA/sgRNA b	96-well	0.25 µg /0.25 µg
	48-well	0.5 µg /0.5 µg
	24-well	1.25 µg /1.25 µg
	12-well	3.75 µg /3.75 µg
	6-well	5 µg / 5 µg
Reagent B (for Mouse Immunocyte CRISPRMax)	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 CRISPRMax)	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	2×105 ~ 3×105 (20 µL)
	48-well	4×105 ~ 6×105 (40 µL)
	24-well	1×106 ~ 1.5×106 (100 µL)
	12-well	3×106 ~ 4.5×106 (300 µL)
	6-well	4×106 ~ 6×106 (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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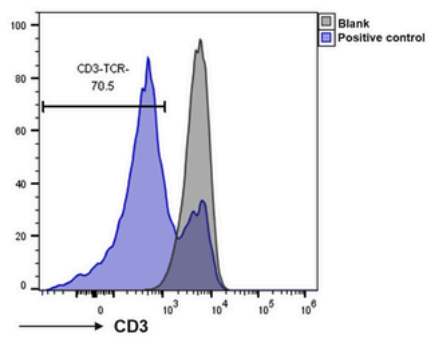
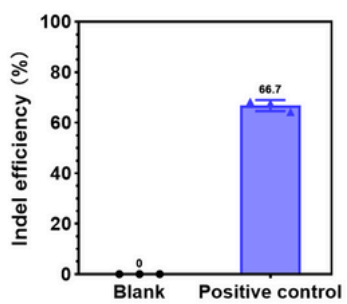
Supporting Data





- **Cell Type:** Mouse primary T Cells
- **Target:** mouse *Trac* gene (sgRNA positive control included in the kit)
- **Detection Time:** 72 hours post-transfection
- **Detection Method**: Flow cytometry analysis of TCR protein expression reveals that approximately 70% 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 66% (see the right figure).

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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.

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: aurorabohsales@gmail.com