Product Overview

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™ CRISPR-Cas9 mRNA Gene Editing Kit is specifically designed for effective gene editing by delivering Cas9 mRNA and sgRNA into difficult-to-transfect cell lines. Additionally, it excels in co-transfecting sgRNAs targeting different genes, enabling simultaneous knockout of multiple genes.

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 human *TRAC* gene.

Table 1 Storage Conditions for the Components

Component	Storage
Reagent A (for CRISPR-Cas9 mRNA)	2-8˚C
Reagent B (for CRISPR-Cas9 mRNA)	-20˚C
EGFP mRNA (1 μg/μL)	-80˚C
Human *TRAC* -sgRNA (1 μg / μL)	-80˚C

Note: Avoid repeated freeze-thaw cycles of Reagent B, EGFP mRNA, and Human *TRAC* -sgRNA.

The targeting sequence of human *TRAC*-sgRNA is TGTGCTAGACATGAGGTCTA.

Pre-Experimental Preparation

Cell Condition: Ensure cells are in optimal physiological condition on the day of transfection, with >90% viability.

Reagent: Allow Reagents A (for CRISPR-Cas9 mRNA) and B (for CRISPR-Cas9 mRNA) to reach room temperature. Briefly mix each reagent 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 Cell Lines
1. Transfection Complex Preparation [a]	
1.1 Mix Reagent A (for CRISPR-Cas9 mRNA) with mRNA	Mix 0.25 μg Cas9 mRNA and 0.25 μg sgRNA with 40 μL of Reagent A (for CRISPR-Cas9 mRNA). **Note:** Invert the Reagent A (for CRISPR-Cas9 mRNA) briefly before use to ensure uniformity.
1.2 Add Reagent B (for CRISPR-Cas9 mRNA)	Add 1.4 μL of Reagent B (for CRISPR-Cas9 mRNA) to the mixture. Mix gently by pipetting up and down 20-30 times or vortexing for 10 seconds.
2. Cell Preparation	
2.1 Suspension cells	Harvest the cells by centrifugation at 300 *g* for 5 minutes. Discard the supernatant and wash cells once with Opti-MEM. Resuspend cells with Opti-MEM and adjust concentration to 5×10^6 - 1×10^7 cells/mL. **Note:** Avoid including FBS in the transfection medium.
2.2 Adherent cells	Maintain 50%-80% cell confluence. Remove medium, wash cells once with Opti-MEM, then add 20 μL of Opti-MEM. **Note:** Avoid including FBS in the transfection medium. **Optional:** Harvest cells by trypsinization, then resuspend them in Opti-MEM at a concentration of 5×10^6 - 1×10^7 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 directly to the cells.
3.2 Incubation	Incubate the cells with the transfection complex for 45-60 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.** 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]	Cell Lines
Reagent A (for CRISPR-Cas9 mRNA)	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 CRISPR-Cas9 mRNA)	96-well	1.4 μL
	48-well	2.8 μL
	24-well	7 μL
	12-well	21 μL
	6-well	28 μL
Recommended Cell Number (Opti-MEM) [c]	96-well	1×10^5 ~2×10^5 (20 μL)
	48-well	2×10^5 ~4×10^5 (40 μL)
	24-well	5×10^5 ~1×10^6 (100 μL)
	12-well	1.5×10^6 ~3×10^6 (300 μL)
	6-well	2×10^6 ~4×10^6 (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.

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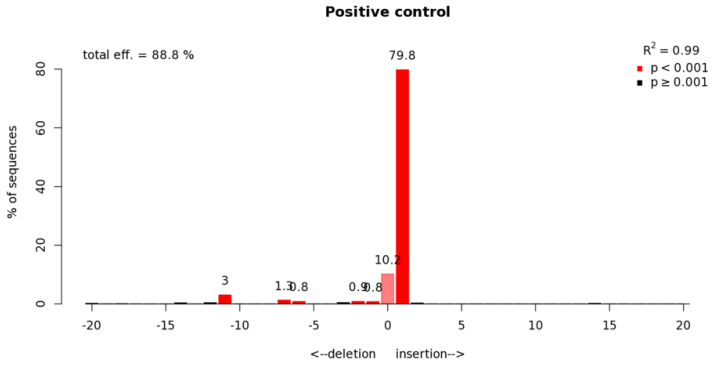
Supporting Data



- **Cell Type:** Jurkat T Cells
- **Target:** human *TRAC* gene (sgRNA positive control included in the kit)
- **Detection Time:** 72 hours post-transfection
- **Detection Method**: Following transfection, genomic DNA was extracted, and the target region was PCR amplified (forward primer sequence: GCAGTATTATTAAGTAGCCCT, reverse primer sequence: AACAAGGCTCACTGTTTCTT). Sanger sequencing results, analyzed by TIDE, reveal that the average editing ratio in the total cell population was about 88%.

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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. **Extend incubation time**: Adjust the incubation time of the transfection complex with cells. The maximum incubation time is 2 hours for cell lines. **Increase ProteanFect transfection complex**: Consider increasing the amount of transfection complex to improve transfection efficiency.

1.2 Improve Cell Condition

For cell lines, transfect cells with >90% viability, confirmed by trypan blue exclusion. Avoid using cells beyond 15 passages, and allow 2-3 passages for recently thawed cells to stabilize before transfection.

1.3 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 should 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.