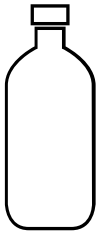
September 2025

ProteanFect™ TuffCell Transfection Kit



0.02 mL (cat. no. TT300007S)
0.1 mL (cat. no. TT300007M)
1.0 mL (cat. no. TT300007L)

A non-viral, non-electroporation, non-liposomal transfection kit using engineered mammalian proteins, tailored for high-efficiency delivery into a wide range of primary cells and difficult to transfect cell lines such as immune cells, stem cells, and neuronal cells.

RUO

For Research Use Only

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REF

TT300007S, TT300007M, TT300007L

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Product Overview

ProteanFect™ TuffCell 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. Specifically developed for hard-to-transfect cell lines and challenging primary cells, the kit ensures robust performance across a broad range of cell types (refer to Table 4). Additionally, it is easily scalable for large-scale experiments and ideal for high-throughput applications.

Component Description

The kit is shipped on dry ice. Once received, store the components as indicated below. The kit includes control payloads EGFP-encoding mRNA (~1000nt) and plasmid DNA (~7kb) to verify transfection efficiency.

Table 1. Storage Conditions for the Components

Component	Storage
Reagent A	2-8°C
Reagent B	-20°C
Reagent C	2-8°C
EGFP mRNA (1 ug/uL)	-20°C
EGFP pDNA (0.5 ug/uL)	-80°C

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 and plasmids.

In Preparation:

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

Reagent: Allow Reagents A, B, and C to reach room temperature before use. Briefly mix thoroughly each reagent by gently inverting or brief vortexing before use.

Nucleic acids: High-purity nucleic acids are essential for efficient transfection. All nucleic acids should be dissolved in nuclease-free water free of salts, and the final concentration adjusted to 0.5–2 μg/μL before use. Plasmid DNA should be prepared using an endotoxin-free purification kit and exhibit an OD260/280 ratio between 1.7 and 1.9. Endotoxin may markedly decrease transfection efficiency.

Transfection Medium: Fetal bovine serum (FBS) or other serum markedly impairs transfection, and additional proteins or excess salt ions may also interfere. Pre-warm the medium to 37°C or room temperature before use.

Recommended media:

- Opti-MEM (preferred)
- Serum-free RPMI 1640 or DMEM (alternatives)

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 Cell Lines	Instructions for Primary Cells [a]
1. Transfection Complex Preparation [b]		
1.1. Mix Reagent A with the payload	Mix the required amount of nucleic acids (see Table 3; 0.5 µg DNA or mRNA per well of a 96-well plate) with 40 µL Reagent A. Briefly invert the tube before use to ensure reagent uniformity.	
1.2 Add Reagent B	Add Reagent B as specified in Table 3 (i.e. 1 µL Reagent B for DNA or 1.4 µL Reagent B for mRNA). Mix thoroughly by pipetting up and down 20–30 times or vortexing for 10 s. Place the transfection complex on ice before adding to cells. **Note:** Thorough mixing is essential for optimal performance.	Add 0.7 µL Reagent B to the mixture. Mix thoroughly by pipetting 20–30 times or vortexing for 10 s. Place the transfection complex on ice before adding to cells. **Note:** Thorough mixing is essential for optimal performance.
1.3 Add Reagent C	**Optional:** Add 8 µL of Reagent C and mix gently by pipetting 2–3 times or vortexing for 2–3 s. Reagent C is not required for most cell lines but may improve efficiency in hard-to-transfect types.	Add 8 µL of Reagent C to the mixture. Mix gently by pipetting up and down 2-3 times or vortexing for 2-3 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:** 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. Ensure the transfection medium contains no FBS or serum.	
3. Transfection		
3.1 Mix transfection complex with cells	For suspension transfection, mix the transfection complex with 20 µL of cell suspension in an Eppendorf tube and gently pipet up and down 2-3 times. For adherent transfection, apply directly to the seeded cells.	
3.2 Incubation	With Reagent C: 15–30 min in incubator. Without Reagent C: Incubate the tube containing cells and the transfection complex for 30–60 min in a cell culture incubator.	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 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). For adherent transfection, replenish with ≥200 µL of complete culture medium.	
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 human primary T cells, which should be stimulated with anti-CD3/CD28 beads or antibodies for 2-10 days 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]	Cell Lines			Primary Cells	
Reagent A	96-well	40 uL				
	48-well	80 uL				
	24-well	200 uL				
	12-well	600 uL				
	6-well	800 uL				
Nucleic Acids [b]		**DNA**	**mRNA**	**siRNA**	**mRNA**	**siRNA**
	96-well	0.2-0.5 ug	0.2-1.0 ug	20-40 pmol	0.2-1.0 ug	20-40 pmol
	48-well	0.4-1.0 ug	0.4-2.0 ug	40-80 pmol	0.4-2.0 ug	40-80 pmol
	24-well	1.0-2.5 ug	1.0-5.0 ug	100-200 pmol	1.0-5.0 ug	100-200 pmol
	12-well	3.0-7.5 ug	3.0-15.0 ug	300-600 pmol	3.0-15.0 ug	300-600 pmol
	6-well	4.0-10.0 ug	4.0-20.0 ug	400-800 pmol	4.0-20.0 ug	400-800 pmol
Reagent B	96-well	1 µL	1.4 µL	1.4 µL	0.7 µL	
	48-well	2 µL	2.8 µL	2.8 µL	1.4 µL	
	24-well	5 µL	7 µL	7 µL	3.5 µL	
	12-well	15 µL	21 µL	21 µL	10.5 µL	
	6-well	20 µL	28 µL	28 µL	14 µL	
Reagent C	96-well	NA			8 µL	
	48-well				16 µL	
	24-well				40 µL	
	12-well				120 µL	
	6-well				160 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.**

Table 4. Primary Cells and Cell Lines Successfully Transfected Using ProteanFect™ TuffCell Transfection Kit

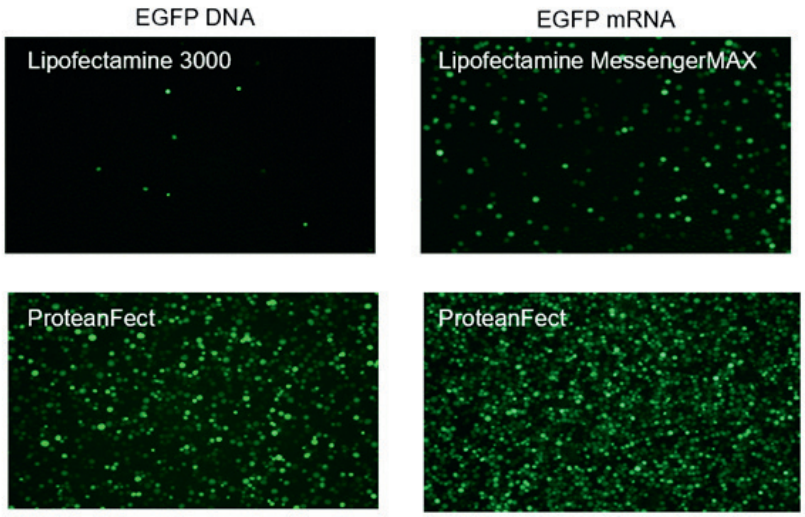
Cell Type		Tested Nucleic Acids	Transfection Efficiency
Cell Lines	Human T Cells	mRNA, siRNA	50-100%
	Human B Cells	mRNA, siRNA	50-75%
	Human Natural Killer Cells	mRNA, siRNA	25-75%
	iPSCs (Induced Pluripotent Stem Cells)	pDNA, mRNA, siRNA	50-100%
	HSCs (Human CD34+ Hematopoietic Stem Cells)	mRNA, siRNA	50-100%
	UC-MSCs (Umbilical Cord Mesenchymal Stem Cells)	mRNA, siRNA	50-100%
	Human Skin Fibroblasts	pDNA, mRNA, siRNA	50-100%
	KSL (Mouse Hematopoietic Stem Cells)	mRNA, siRNA	50-100%
	Mouse Neurons	pDNA, mRNA, siRNA	10-50%
	Mouse Oligodendrocytes	pDNA, mRNA, siRNA	50-100%
	Porcine Macrophages	mRNA, siRNA	50-75%
	Bovine Fibroblasts	mRNA, siRNA	50-100%
	M. rosenbergii Hemocytes	mRNA, siRNA	25-75%
	Chicken Primordial Germ Cells	mRNA, siRNA	25-100%
	L. crocea Mesenchymal Stem Cells	mRNA, siRNA	25-50%
	Jurkat (Human T Cells)	pDNA, mRNA, siRNA	50-100%
	MOLT-16 (Human T-ALL Cells)	mRNA, siRNA	10-30%
	Raji (Human B Cells)	mRNA, siRNA	50-100%
	MEC-1 (Human B Cells)	mRNA, siRNA	50-100%
	TMD8 (Human Diffuse Large B-Cell Lymphoma Cells)	mRNA, siRNA	50-100%
	NK-92 (Human Natural Killer Cells)	mRNA, siRNA	25-75%
	K562 (Human Chronic Myeloid Leukemia)	pDNA, mRNA, siRNA	30-100%
	THP-1 (Human Monocytic Cells)	mRNA, siRNA	50-100%
	Kasumi-1 (Human Acute Myeloid Leukemia)	mRNA, siRNA	50-100%
	MDS-L (Human Leukemia Cells)	mRNA, siRNA	50-100%
	U937 (Human Myeloid Leukemia)	mRNA, siRNA	50-75%
	HL-60 (Human Promyelocytic Leukemia)	mRNA, siRNA	50-100%
	HFF (Human Fibroblasts)	mRNA, siRNA	50-100%
	LX-2 (Human Hepatic Stellate Cells)	pDNA, mRNA, siRNA	50-100%
	HepG2 (Human Liver Carcinoma)	pDNA, mRNA, siRNA	25-75%
	KYSE-510 (Human Esophageal Squamous Cell Carcinoma)	mRNA, siRNA	50-100%

Table 4. Primary Cells and Cell Lines Successfully Transfected Using ProteanFect™ TuffCell Transfection Kit

Cell Type		Tested Nucleic Acids	Transfection Efficiency
Cell Lines	Mum2B (Human Melanoma)	mRNA, siRNA	50-100%
	H1-hESC (Human Embryonic Stem Cells)	pDNA, mRNA, siRNA	50-100%
	U2OS (Human Osteosarcoma)	pDNA, mRNA, siRNA	50-100%
	HT-29 (Human Colorectal Adenocarcinoma Cells)	pDNA, mRNA, siRNA	50-100%
	5637 (Human Bladder Carcinoma Cells)	pDNA, mRNA, siRNA	50-100%
	HGC-27 (Human Gastric Carcinoma Cells)	mRNA, siRNA	50-100%
	SH-SY5Y (Human Neuroblastoma Cells)	pDNA, mRNA, siRNA	50-100%
	U251MG Cells (Human Glioblastoma Cells)	mRNA, siRNA	50-100%
	HEK293 (Human Embryonic Kidney Cells)	pDNA, mRNA, siRNA	50-100%
	Neuro 2A (Mouse Neuroblastoma Cells)	pDNA, mRNA, siRNA	50-100%
	MC38 (Mouse Colon Adenocarcinoma Cells)	mRNA, siRNA	25-75%
	RAW264.7 (Mouse Macrophages)	mRNA, siRNA	25-50%
	LLC (Mouse Lewis Lung Carcinoma)	pDNA, mRNA, siRNA	50-100%
	CH12 (Mouse Lymphoma Cells)	mRNA, siRNA	50-100%
	BV-2 (Mouse Microglial Cells)	mRNA, siRNA	25-50%
	C2C12 (Mouse Myoblasts)	pDNA, mRNA, siRNA	50-100%
	B16 (Mouse Melanoma Cells)	mRNA, siRNA	25-75%
	MH-S (Mouse Macrophages)	mRNA, siRNA	25-50%
	MODE-K (Mouse Intestinal Epithelial Cells)	mRNA, siRNA	50-100%
	MEF (Mouse Embryonic Fibroblasts)	pDNA, mRNA, siRNA	25-100%
	3T3 (Mouse Embryonic Fibroblasts)	mRNA, siRNA	25-50%
	OLN93 (Rat Oligodendrocyte Cells)	mRNA, siRNA	50-100%
	MDBK (Bovine Kidney Epithelial Cells)	mRNA, siRNA	50-100%
	MAC-T (Bovine Mammary Epithelial Cells)	mRNA, siRNA	50-100%
	COS-7 (Monkey Kidney Fibroblast-like Cells)	pDNA, mRNA, siRNA	50-100%

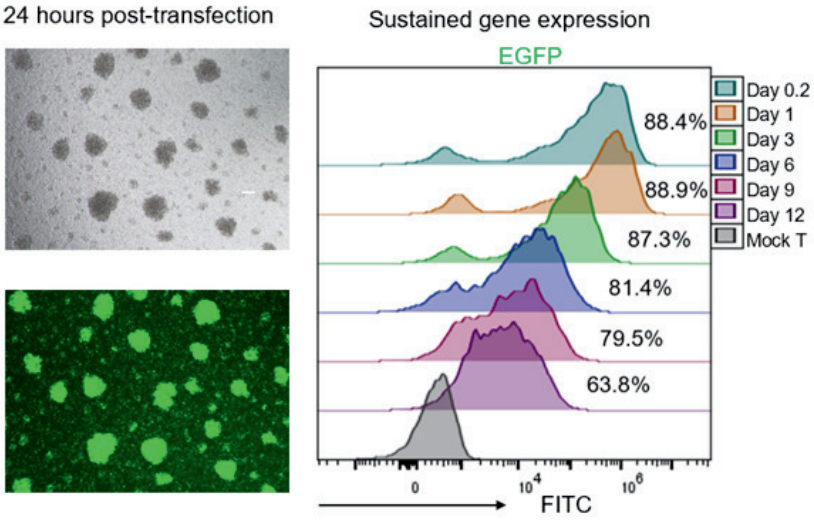
Application Data:

Case 1: Successful Transfection of Jurkat T Cells



- **Cell Type:** Jurkat T Cells
- **Transfected Nucleic Acid Type:** EGFP pDNA or EGFP mRNA
- **Detection Time:** 48 hours post-pDNA transfection; 24 hours post-mRNA transfection

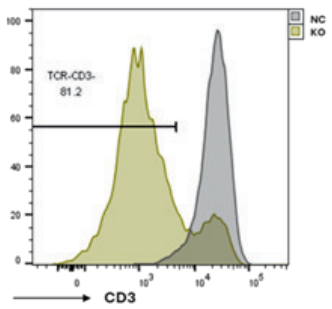
Case 2: Successful Transfection of primary human T Cells

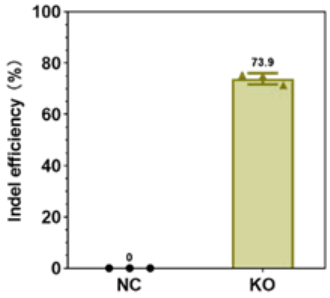


- **Cell Type:** Primary human T cells continuously activated for 6 days using Dynabeads™ Human T-Expander CD3/CD28 (Thermo Fisher, 11141D).
- **Transfected Nucleic Acid Type:** EGFP mRNA.
- **Detection Time:** Continuous monitoring from 5 hours to 12 days post-transfection.

Application Data:

Case 3: Successful gene knockout of primary human T cells





- **Cell Type:** Human primary T cells
- **Transfection Complex:** Reagent A, B, C with Cas9 protein and sgRNA targeting human *TRAC* gene
- **Detection Time:** 72 hours post-transfection
- **Detection Method**: Flow cytometry analysis of TCR protein expression reveals that approximately 81% of T cells in the KO group were CD3-TCR complex negative (see the left figure). Sanger sequencing following PCR amplification of the target region (forward primer sequence: GCAGTATTATTAAGTAGCCCT, reverse primer sequence: AACAAGGCTCACTGTTTCTT), analyzed by TIDE, reveals that the average editing ratio in the total cell population was about 74% (see the right figure).

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 2 hours for cell lines, and 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 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.

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