# PT02-ProteanFect™ Max Transfection Kit: Instructions for Use

## Product Overview

ProteanFectTM Max 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 positive control samples with EGFP-encoding mRNA and plasmid DNA (pDNA) 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 μg/μL) | -80°C |
| GFP pDNA (0.5 μg/μL) | -20°C |

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

## Pre-Experimental 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. 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 2Transfection 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 mRNA | Mix 0.5 μg of mRNA with 40 μL of Reagent A.<br>**Note:** Invert Reagent A briefly before use to ensure uniformity. | |
| 1.2 Add Reagent B | Add 1.4 μL of Reagent B to the mixture. Mix gently by pipetting up and down 20-30 times or vortexing for 10 seconds. | Add 0.7 μL of Reagent B to the mixture. Mix gently by pipetting up and down 20-30 times or vortexing for 10 seconds. |
| 1.3 Add Reagent C | N/A | 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 $5\times10^6$ - $1\times10^7$ cells/mL.<br>**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.<br>**Note:** Avoid including FBS in the transfection medium.<br>**Optional:** Harvest cells by trypsinization, then resuspend them in Opti-MEM at a concentration of $5\times10^6$ - $1\times10^7$ cells/mL for subsequent transfection. | |
| **3. Transfection** | | |
| 3.1 Mix 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. | 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).<br>**Note:** The cell pellet may adhere to the tube walls. Gently remove the supernatant to minimize cell loss. | |
| 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. | |

FBS, Fetal bovine serum. **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.


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**Table 3 Transfection Guidelines for Different Culture Formats**

| Components | Culture Vessels a | Cell Lines | | | Primary Cells | |
|---|---|---|---|---|---|---|
| **Reagent A** | 96-well | 40 µL | | | | |
| | 48-well | 80 µL | | | | |
| | 24-well | 200 µL | | | | |
| | 12-well | 600 µL | | | | |
| | 6-well | 800 µL | | | | |
| **Nucleic Acids b** | | **DNA** | **mRNA** | **siRNA** | **mRNA** | **siRNA** |
| | 96-well | 0.4 µg | 0.5 µg | 40 pmol | 0.5 µg | 20 pmol |
| | 48-well | 0.8 µg | 1 µg | 80 pmol | 1 µg | 40 pmol |
| | 24-well | 2 µg | 2.5 µg | 200 pmol | 2.5 µg | 100 pmol |
| | 12-well | 6 µg | 7.5 µg | 600 pmol | 7.5 µg | 300 pmol |
| | 6-well | 8 µg | 10 µg | 800 pmol | 10 µg | 400 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 | 1×105 ~2×105 (20 µL) | | | | |
| | 48-well | 2×105 ~4×105 (40 µL) | | | | |
| | 24-well | 5×105 ~1×106 (100 µL) | | | | |
| | 12-well | 1.5×106 ~3×106 (300 µL) | | | | |
| | 6-well | 2×106 ~4×106 (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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**Table 4 Primary Cells and Cell Lines Successfully Transfected Using ProteanFect™ Max Transfection Kit**

| Cell Lines | | Tested Nucleic Acid Types for Transfection |
|---|---|---|
| Primary cells | Human primary T cells | mRNA, siRNA |
| | Human primary NK cells | mRNA, siRNA |
| | Human primary monocytes | mRNA, siRNA |
| | Human primary CD34+ hematopoietic stem cells | mRNA, siRNA |
| | Mouse primary cardiomyocytes | mRNA, siRNA |
| | Mouse primary neurons | pDNA, mRNA, siRNA |
| | Mouse primary glial cells | pDNA, mRNA, siRNA |
| | Mouse primary T cells | mRNA, siRNA |
| | Mouse primary NK cells | mRNA, siRNA |
| | Large yellow croaker primary mesenchymal stem cells | mRNA, siRNA |
| Cell lines | Jurkat T (human T lymphoblastic leukemia cells) | pDNA, mRNA, siRNA |
| | LX-2 (human hepatic stellate cells) | pDNA, mRNA, siRNA |
| | HepG2 (human liver tumor cells) | pDNA, mRNA, siRNA |
| | THP-1 (human acute monocytic leukemia cell line) | mRNA, siRNA |
| | Raji (human Burkitt's lymphoma cells) | mRNA, siRNA |
| | K562 (human chronic myeloid leukemia cells) | pDNA, mRNA, siRNA |
| | MOLT-16 (human T lymphoblastic leukemia cells) | mRNA, siRNA |
| | SH-SY5Y (human neuroblastoma cells) | pDNA, mRNA, siRNA |
| | U2OS (human osteosarcoma cells) | pDNA, mRNA, siRNA |
| | U937 (human lymphoma cell line) | mRNA, siRNA |
| | HFF (human foreskin fibroblasts) | pDNA, mRNA, siRNA |
| | HEK-293 (human embryonic kidney cell line) | pDNA, mRNA, siRNA |
| | MC38 (mouse colon cancer cells) | mRNA, siRNA |
| | RAW264.7 (mouse mononuclear macrophage leukemia cells) | mRNA, siRNA |
| | LLC (mouse Lewis lung cancer cells) | pDNA, mRNA, siRNA |
| | C2C12 (mouse myoblasts) | pDNA, mRNA, siRNA |
| | COS7 (African green monkey kidney fibroblast-like cells) | pDNA, mRNA, siRNA |
| | | |


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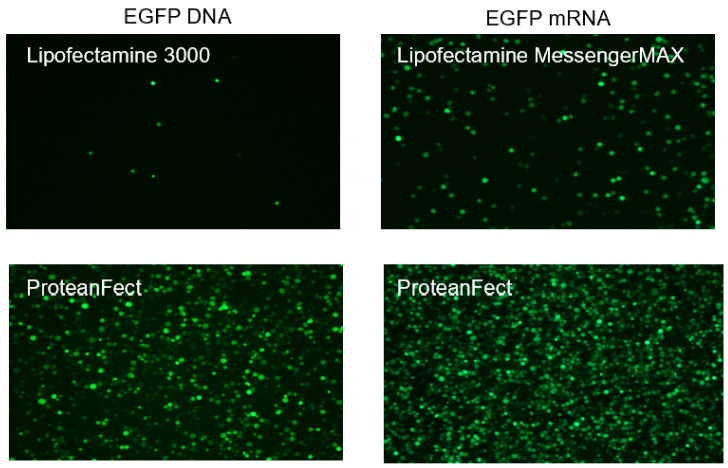
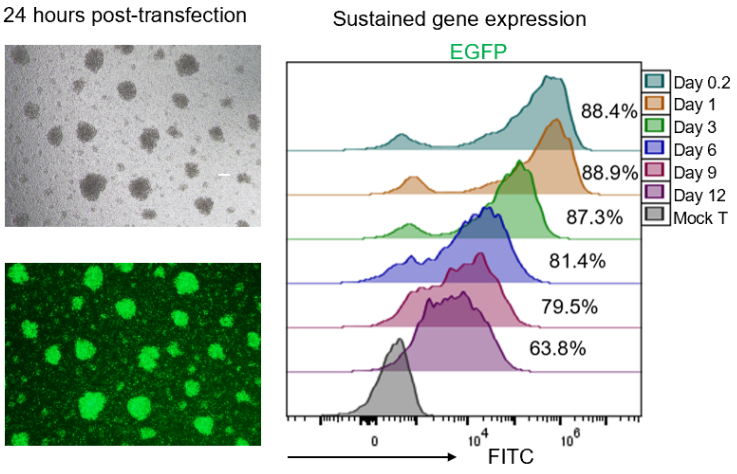
## Supporting Data

**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.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# PT02-ProteanFect Max™ Transfection Kit: Instructions for Use

## 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 Improve Plasmid DNA Quality

Transfection efficiency is highly dependent on the quality of the plasmid DNA. **Endotoxin-free**: Use an endotoxin-free kit to prepare plasmid DNA. **Optimal concentration**: Ensure an OD260/280 ratio between 1.7 and 1.9, and dilute DNA to 0.5–2 μg/μL using nuclease-free water.

#### 1.4 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.

**For primary cells,** proper activation is crucial for optimal transfection efficiency. For example, human primary T cells generally achieve the best transfection results after stimulation with anti-CD3/CD28 activation beads or antibodies for 2-10 days, with peak efficiency typically observed around days 4-6.

#### 1.5 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


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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.