# PT03-ProteanFect™ Max Mouse Immunocyte Transfection Kit: Instructions for Use

## Product Overview

ProteanFectTM 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 γδ T cells, the kit ensures reliable performance with 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 to verify transfection efficiency.

**Table 1 Storage Conditions for the Components**

| Component | Storage |
|---|---|
| Reagent A (for Mouse Immunocyte) | 2-8°C |
| Reagent B (for Mouse Immunocyte) | -20°C |
| Reagent C (for Mouse Immunocyte) | 2-8°C |
| EGFP mRNA (1 μg/μL) | -80°C |

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

## 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) 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 2Transfection 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) with mRNA | Mix 0.5 μg of mRNA with 40 μL of Reagent A (for Mouse Immunocyte).<br>**Note:** Invert Reagent A (for Mouse Immunocyte) briefly before use to ensure uniformity. |
| 1.2 Add Reagent B (for Mouse Immunocyte) | Add 0.7 μL of Reagent B (for Mouse Immunocyte) to the mixture. Mix gently by pipetting up and down 20-30 times or vortexing for 10 seconds. |
| 1.2 Add Reagent C (for Mouse Immunocyte) | Add 10 μL of Reagent C (for Mouse Immunocyte) to the mixture. Mix gently by pipetting up and down 2-3 times or vortexing for 2-3 seconds.<br>**Note:** If precipitation occurs in Reagent C (for Mouse Immunocyte), 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.<br>**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.<br>**Note:** Avoid including FBS in the transfection medium.<br>**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).<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 the 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 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)** | 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 | 0.5 µg | 20 pmol |
| | 48-well | 1 µg | 40 pmol |
| | 24-well | 2.5 µg | 100 pmol |
| | 12-well | 7.5 µg | 300 pmol |
| | 6-well | 10 µg | 400 pmol |
| **Reagent B (for Mouse Immunocyte)** | 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)** | 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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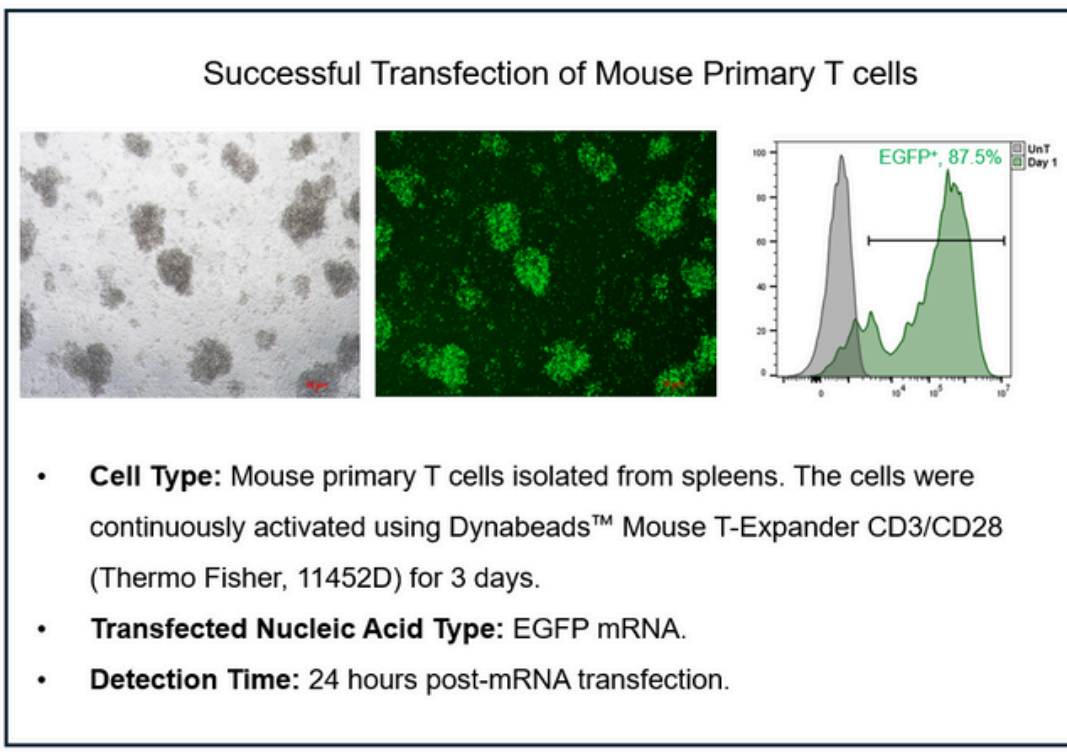
## Supporting Data




- **Cell Type:** Mouse primary T cells 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.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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.