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# Protein Transfection Reagent

## 1 Components

| Components | HY-K2016-0.3 mL | HY-K2016-1.5 mL |
|---|---|---|
| Protein Transfection Reagent | 0.3 mL | 0.3 mL × 5 |

## 2 Introduction

MCE Protein Transfection Reagent is a cationic lipid mixture for complexation with proteins, peptides, antibodies and other biologically active molecules to allow their direct intracellular delivery. Reagent protein complexes attach to negatively charged cell surfaces and either directly fuses with the membrane to deliver the captured protein into the cell or be endocytosed by the cell and then fuse with the endosome, releasing the captured protein into the cytoplasm.

Features of MCE Protein Transfection Reagent

· Fast protein delivery: Optimum delivery in 3 to 12 h after incubation, with high transfection efficiency for primary cells and difficult-to-transfect cells.
· High protein activity: Delivery complex is non-covalent, allowing proteins and peptides retain biological activity after delivery.
· Versatility: Suitable for a wide range of cells, works with proteins, peptides and fluorescently labeled antibodies.
· Low cytotoxic: It exhibits mild action and is biocompatible.
· Time-saving: Eliminates the need to isolate, clone and transfect gene sequences.

## 3 Flow Diagram of Experiment

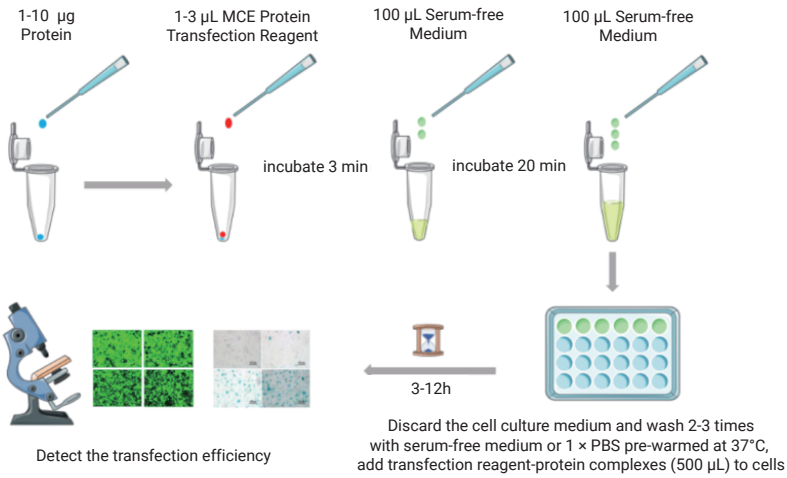


**Figure 1. The process of the Protein Transfection**

## 4 General Protocol

This is the protocol for transfection in the 24-well plate, and the adding volume of other culture devices is shown in Table 1.

1.Prepare cells

(1)For adherent cells: Plate the cells digested with trypsin one day before transfection (1-3 × $10^5$ cells plated in a 24-well cluster plate), until the density reaches 50%-100% for cell transfection.

(2)Foe suspension cells: Plate the cells before transfection and suspend in fresh medium (2-6 × $10^5$ cells/500 μL medium).

Note: The viability and general health of cells prior to transfection significantly affect the transfection result. Cells should be at least 90% viable prior to transfection and have had sufficient time to recover from passaging.

2.Prepare Protein Transfection Reagent/ Protein complexes

(1)Add 1-10 μg protein to a 1.5 mL EP tube, and add 1-3 μL MCE Protein Transfection Reagent, mix gently and incubate for 3 min at room temperature.

Note: The amount of protein should be optimized according to the protein and experiment type. The optimal transfection conditions vary depending on the cell type and culture conditions (e.g. 5-10 μg of fluorescent protein GFP, 0.5-2 μg of fluorescent protein Phycoerythrin, 0.1-1 μg of apoptotic protein Saporin, etc.), perform pre-experiment to determine the optimal transfection ratio. The volume of transfection reagent should be adjusted according to the protein concentration and the size of the petri dish, please refer to Table 1.

**Table 1 Protein Transfection Reagent: Serum-free Medium Ra**

| Plate Size | Growth Medium (mL) | DNA (μg) | Lentivirus Transfection Reagent (μL) | Serum-freeMedium (μL) |
|---|---|---|---|---|
| 96-well | 0.1 | 0.2-0.6 | 20 | 80 |
| 24-well | 0.5 | 1-3 | 100 | 400 |
| 12-well | 1 | 2-6 | 200 | 800 |
| 6-well | 2 | 4-12 | 400 | 1,600 |
| 60 mm | 4 | 8-24 | 800 | 3,200 |
| 100 mmL | 10 | 20-60 | 2,000 | 8,000 |

(2)Add 100 μL Serum-free Medium1, mix gently and incubate for 20 minutes at room temperature.

(3)Add 400 μL Serum-free Medium2 and mix gently to dilute the transfection reagent-protein complexes.

3.Cell Transfection

During incubation of the transfection reagent-protein complexes, discard the cell culture medium and wash 2-3 times with serum-free medium or 1 × PBS pre-warmed at 37°C. Add transfection reagent-protein complexes (500 μL) to each well, mix gently and incubator for further culture.

4.Transfection efficiency analysis

After incubation of 3-12 h, the transfection effect can be analyzed by fluorescence detection, Western Blot, ELISA, RT-PCR, flow cytometry, reporter gene, immunofluorescence staining and other methods according to the experimental needs.

Note: After 3 hours of incubation, replenish the transfection solution or discard the transfection solution and replace it with complete medium (optional); Before re-examination or visualization, wash cells 3-4 times with serum-free medium or 1 × PBS pre-warmed at 37°C to remove the untransfected proteins.

## 5 Storage

-20°C，1 year

Avoid repeat freeze-thaw cycles.

## 6 Precautions

1.Use high-purity and high-activity proteins for transfection to obtain high transfection efficiency.

2.The viability and general health of cells prior to transfection significantly affect the transfection result. Cells should be at least 90% viable prior to transfection and have had sufficient time to recover from passaging.

3.To improve transfection efficiency, Serum-free Medium is recommended.

4.The time of cells exposed to the transfection complexes will affect transfection efficiency. The optimal protein delivery time needs to be determined by mapping. In general, peptides and small proteins enter the cell at about 2 h, most enzymes and medium-sized proteins are transferred into the cell at 3-3.5 h, and large proteins, antibodies or multimeric proteins require 5 h or more for efficient delivery.

5.This product is for R&D use only, not for drug, household, or other uses.

6.For your safety and health, please wear a lab coat and disposable gloves to operate.