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siRNA/miRNA Transfection Reagent

1 Components

Components	HY-K2017-1 mL	HY-K2017-5 mL
siRNA/miRNA Transfection Reagent	1 mL	1 mL × 5

2 Introduction

RNA interference (RNAi) refers to the small double-stranded RNAs that can specifically degrade homologous mRNAs, inhibiting or shutting down the expression of the corresponding genes. By utilizing RNAi technology to design small interfering RNA (siRNA) targeting the mRNA of disease-causing genes, it is possible to specifically knock out or turn off the expression of specific genes.

MCE siRNA/miRNA Transfection Reagent is a novel cationic polymer transfection reagent for siRNA and miRNA transfection. The exogenous siRNA/miRNA forms a stable complex with the transfection reagent and enters the cell through cytosis, subsequently releasing the siRNA/miRNA in the cytoplasm to achieve cellular transfection of siRNA/miRNA.

Features of MCE siRNA/miRNA Transfection Reagent

· Wide range of applications: High transfection efficiency in many common cell types, suitable for siRNA/miRNA-mediated gene suppression experiments.

· High transfection efficiency: Efficient transfection of primary cells with high cell viability.

· Low cytotoxicity: Mild action and biocompatible.

· Simple operation: No need to remove transfection complexes or change medium after transfection.

3 Flow Diagram of Experiment

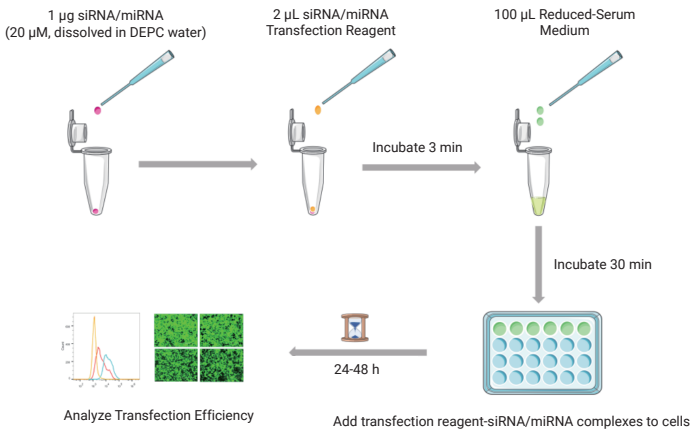

Figure 1. The process of the siRNA/miRNA Transfection

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 (0.1-1 × 10^5 cells plated in a 24-well cluster plate), until the density reaches 30%-40% for cell transfection.

(2)Foe suspension cells: Plate the cells before transfection and suspend in fresh medium (0.5-2.5 × 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 Transfection Reagent complexes

(1)Add 1 μg siRNA/miRNA (20 μM, dissolved in DEPC water) to a 1.5 mL EP tube, and add 2 μL siRNA/miRNA Transfection Reagent, mix gently and incubate for 3 minutes at room temperature.

Note: The optimal transfection conditions vary depending on the cell type and culture conditions, perform pre-experiment to find out the optimal transfection ratio. The volume of transfection reagent should be adjusted according to the siRNA/miRNA concentration and the size of the petri dish, please refer to Table 1.

Table 1 siRNA/miRNA Transfection Reagent: Serum-free Medium Ratio

Plate Size	Growth Medium (mL)	siRNA/miRNA (20 μM) (μL)	siRNA/miRNA Transfection Reagent (μL)	reduced-serum medium (μL)
96-well	0.1	0.2	0.4	20
24-well	0.5	1	2	100
12-well	1	2	4	200
6-well	2	4	8	400
60 mm	4	8	16	800
100 mm	10	20	40	2,000

(2)Add 100 μL reduced-serum medium (serum-free and antibiotic-free), mix gently and incubate for 30 minutes at room temperature.

3.Cell Transfection

Add transfection reagent-siRNA/miRNA complexes (100 μL) to each well, mix gently and incubator for further culture.

4.Analyze transfection efficiency

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.

5 Storage

-20°C，1 year

Avoid repeat freeze-thaw cycles.

6 Precautions

1.To improve transfection efficiency, high purity siRNA/miRNA purified by PAGE and desalted is recommended.

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.Generally, the ratio of siRNA/miRNA (μL) to siRNA/miRNA Transfection Reagent (μL) is 1:2, and the transfection efficiency can be optimized in the range of 1:1 to 1:3 if necessary.

4.During transfection, the presence of antibiotics may lead to reduced transfection efficiency and cytotoxicity, and the addition of antibiotics to the transfection medium is not recommended.

5.To improve transfection efficiency, serum-free Medium is recommended.

6.There are many factors affecting transfection efficiency, such as cell type, cell state and density, nucleic acid quality and concentration, ratio of transfection reagents to nucleic acids, etc. It is recommended to perform pre-experiment to find out the best transfection conditions first.

7.RNA-free enzymes and non-pyrogenic materials were used throughout the experiment.

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

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