Signosis

Innovative Plate Assay Solutions

pMiR-Luc Reporter Vectors

Catalog Number LR-XXXX **(For Research Use Only)**

Introduction

pMiR-Luc™ reporter vectors are a series of firefly luciferase-based reporter constructs for quantitative measurement of miRNA expression in cells. Each vector contains the CMV promoter, firefly luciferase gene, a unique miRNA target site at 3'UTR, and a SV40 terminator sequence. The target site is a sequence perfectly complementary to a specific miRNA. When the miRNA is expressed, it binds to the sequence and results in repression of luciferase gene expression. Therefore, luciferase activity represents the expression and activity of a miRNA. When pMiR-Luc reporter vectors are transfected into mammalian cells, they can be used to detect endogenous miRNA expression and activity, or used to monitor the up- or down-regulation of miRNAs.

Recommend transfection and assay

We recommend using FuGENE™ 6 (Roche) for the transfection of pMiR-Luc reporter vectors, as the use of other transfection methods could lead to reduced luciferase activity from the reporters.

1. Plate 1-3x10^5 cells in 1 ml of growth medium containing serum without antibiotics in a 12-well culture plate at one day before transfection, which will yield 50-80% confluence on the day of transfection. We recommend to plate cells in duplicate.
2. For each transfection, dilute 0.2 µg of the reporter vector with 50 µl of Opti-MEM I Reduced Serum Medium or serum-free culture media and dilute 3 µl FuGENE 6 Reagent with another 50 µl of Opti-MEM I Reduced Serum Medium or serum-free culture media, mix, and incubate for 5 min at room temperature, but no longer. Combine the diluted mix. Incubate for 15-30 min at room temperature. Once the FuGENE 6 Reagent is diluted, it needs to use within 45 min.
3. Add 100 µl of DNA/FuGENE mix to the complete growth media on cells and mix gently by rocking the plate back and forth. Incubate the cells at 37°C in a CO2 incubator, overnight.
4. Measure luciferase expression 24-48 hr after transfection. Aspirate to completely remove the media from the culture plates.
5. Lyse the attached cells by adding lysis buffer to each well. Use approximately 50 µl per well for a 12-well plate. To detach cells from the plate, pipet the mixture up and down. Transfer the cell lysate/buffer solution to a clean 1.5-ml microcentrifuge tube. Keep on ice or store at -20°C. Assay for luciferase activity following the instructions given by the supplier.

E. coli transform to propagate the plasmids

1. Transform *E. coli* competent cells with the plasmid.
2. Plate the transformed cells on LB plates containing 100 µg/mL Ampicillin and grow overnight at 37°C.
3. Transfer a single colony to 1-2 ml LB medium containing 100µg/mL and shake at 37°C overnight.
4. Prepare plasmids and check on gel.

Diagram of pMiR-Luc reporter vectors