Signosis
Innovative Plate Assay Solutions

pMiR-Luc Target Reporter Vectors

Catalog Number LR-1XXX **(For Research Use Only)**

Introduction

MicroRNAs regulate gene expression by targeting messenger RNAs at specific sites to induce the cleavage of the targets or inhibit translation. The interaction of a miRNA with its targets is known mediated by RNA-induced silencing complex (RISC). RISC-mediated interaction between a mature miRNA and its binding site depends on sequence complementarities between miRNA and its targets and the total number of binding sites in a given 3'UTR. Numerous important regulatory genes have been found to be regulated by miRNAs, such as Ras by let-7, Bcl-2 by miR-15 and miR-16, ERa by miR206, TPM1 by miR-21, and PTEN by miR-19a. Luciferase reporter-based cellular assays are often employed to monitor the regulation of these genes by miRNAs. pLuc-3UTR reporter vectors are a series of firefly luciferase-based reporter constructs for monitoring miRNA-mediated regulation of target genes in cells. Each vector contains the CMV promoter, firefly luciferase gene, the 3'UTR (up to 1kb) of a target gene, and a SV40 terminator sequence. When a miRNA is expressed and binds to 3'UTR, it results in repression of luciferase gene expression. Therefore, miRNA-mediated regulation of a specific target can be monitored through luciferase activity. When two samples are compared by measuring the activities of luciferase, the difference can be identified and the regulation dissected.

Recommend transfection and assay

We recommend using FuGENE™ 6 (Roche) for the transfection of pMiR-Luc target 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.
6. 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 100 µg/mL and shake at 37°C overnight.
4. Prepare plasmids and check on gel.

Diagram of pMiR-Target Luc reporter vectors