Signosis
Innovative Plate Assay Solutions

Genotoxic Stress miRNA Reporter Vector Set

Catalog Number LR-4002 **(For Research Use Only)**

Introduction

Exposure to genotoxic agents such as ultraviolet light, oxidative stress, and chemical mutagens leads to DNA damage, which is a major cause of human cancers. Cellular responses are important to effective defense against genotoxic stress, and recent studies have indicated that miRNAs control the expression of target genes at post-transcriptional level in the process.
Signosis has developed a set of miRNA luciferase reporter vectors (miR-34a, miR-107, miR-21, miR-125b, let-7b, miR-149, miR-22 and Negative control) to facilitate genotoix stress study.

Recommend transfection and assay

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

Below is the procedure for each vector:

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 100 µg/mL and shake at 37°C overnight.
4. Prepare plasmids and check on gel.

Diagram of pMiR-Luc reporter vectors