Signosis
Innovative Plate Assay Solutions

Apoptosis Luciferase Reporter Vector Set

Catalog Number LR-3002 **(For Research Use Only)**

Introduction

Apoptosis is known as programmed cell death implicated in biological processes ranging from embryogenesis to ageing and from normal tissue homoeostasis to many human diseases. A number of signaling pathways that lead to characteristic cell changes are involved in mediating the apoptosis responses. One critical change in signaling pathways is the activation of transcription factors (TFs), and TF-based reporters are often used to monitor the activation of signaling pathways.
Signosis has developed a set of luciferase reporter vectors (NFkB, p53, Myc, AP1, IRF, ELK, NFAT, and Negative control) to facilitate apoptosis study.

Recommend transfection and assay

We recommend using FuGENE 6 (Roche) for the transfection of pTF-Luc reporter vectors. For difficult-to -transfect cell type such as primary cells, we recommend using Fugene HD (Roche) for the transfection. The transfection can be done in 6-well or 12 well plates.
The following protocol is designed for adherent cultures in **6-well** plates using FuGENE 6. If you use a different size of plate or flasks, adjust the components in proportion to the surface area of your container.

Below is the assay procedure for each of the 8 vectors:

1. For each of the 8 vectors, plate 1-4x10^5 cells in 2 ml of growth medium containing serum without antibiotics in a 6-well culture plate at one day before transfection, which will yield 50-80% confluence on the day of transfection.

2. dilute 0.5-1 µg of the reporter vector with 100 µl of serum-free culture medium, and in a separate tube, dilute 3 µl FuGENE 6 Reagent with 100 µl of serum-free culture medium (add transfection reagent directly into the medium and don't touch the wall of the tube). Add the diluted reporter vector to the diluted transfection reagent and gently 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 200 µl of DNA/FuGENE complex to on the cells in a drop-wise manner. Evenly distribute the complex by gently rocking the plate back and forth. Incubate the cells at 37^{o}C in a CO2 incubator for overnight.

4. If the starvation is required, replace the medium with serum free or low serum medium (0.2% serum) for 6 -16 hours, and treat the cell with the selected stimulus for 8-14 hours.

5. Alternatively, to study the effects of a gene of interest, cotransfect each pTF-Luc with a gene expression vector of interest.
6. Lyse the attached cells by adding lysis buffer (Promega, Luciferase Assay System) to each well. Use approximately 200 µl per well for a 6-well plate. To detach cells from the plate, freeze and thaw the plate once and pipette the mixture up and down. Transfer the cell lysate/buffer solution to a clean 1.5-ml microcentrifuge tube, which is ready for luciferase assay or store at -80^{o}C for the future use. Assay for luciferase activity following the instructions given by the supplier (Promega, Luciferase Assay System, cat.# E1500).

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^{o}C overnight.
4. Prepare plasmids and check on gel.