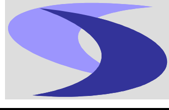


Signosis
Innovative Plate Assay Solutions

pLuc5U-Oxidative Stress Luciferase Reporter Vector Set

Catalog Number LR-5001 **(For Research Use Only)**

Introduction

The excessive production of reactive oxidative species (ROS) leads to a condition of oxidative stress. Oxidative stress can directly damage cell structures and may results in cancer, inflammatory diseases, and aging. iNOS, Gadd153/CHOP, COX-2, HO-1, GPx-1, NRF2, and BCL2 are important inducible mediators during the oxidative stress process. The measurement of the changes in transcriptional regulation of these genes is vital to monitoring the oxidative stress status and deciphering the underlying mechanisms of this critical cellular function in response to a wide range of cellular stresses. Signosis has developed a set of gene promoter luciferase reporters for these genes including iNOS, Gadd153/CHOP, COX-2, HO-1, GPx-1, NRF2, and BCL2. These vectors contain the reporter luciferase gene downstream of the selected promoter region of each gene, which functional testing has been well documented.

Recommended transfection and assay

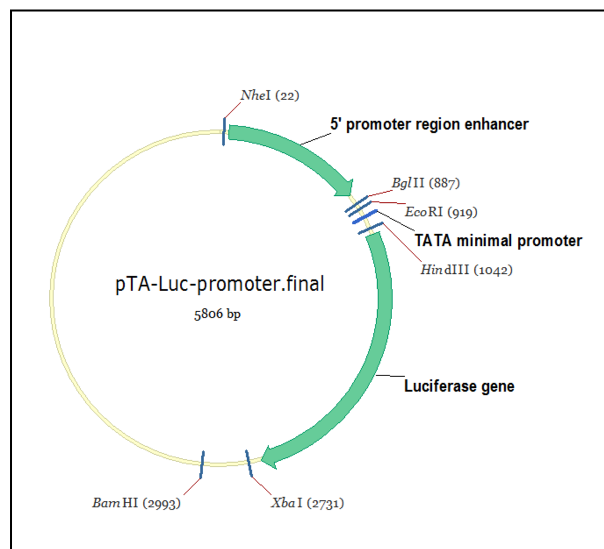
We recommend using FuGENE 6 (Roche) for the transfection of pLuc5U reporter vectors. For difficult-to -transfect cell type such as primary cells, we recommend using Fugene HD (Roche) for the transfection. The transfection can be done in 6-well or 12 well plates.
The following protocol is designed for 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.

1. 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. For each transfection, 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 do not 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 be used 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°C in a CO_2 incubator overnight.
4. If 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 pLuc5U 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 can be stored at -80°C for the future use. Assay for luciferase activity following the instructions given by the supplier (Promega, Luciferase Assay System, cat.# E1500).

E. coli transformation 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 of LB medium containing 100 µg/ml Ampicillin and shake at 37°C overnight.
4. Prepare plasmids and verify on gel.

Diagram of pLuc5U reporter vectors



Signosis, Inc. • 1700 Wyatt Drive Suite 10-12 • Santa Clara, CA 95054 • Tel 408 747 0771 • Fax 408 864 2182