



Antioxidant Luciferase Reporter Vector Set

Catalog Number LR-3003

(For Research Use Only)

Introduction

Reactive oxygen species (ROS) are critical to cell proliferation, growth arrest, and apoptosis. ROS comes mostly from the endogenous metabolic by-products and also from exogenous sources such as exposure to cigarette smoke, environmental pollutants, and bacterial, fungal or viral infections. Excess ROS must be promptly eliminated from the cell by different antioxidant defense mechanisms, in order to reduce oxidative that has been implicated in the cause of many diseases, such as cancer, Alzheimer's disease, Parkinson's disease, atherosclerosis, and heart failure. Cellular antioxidant enzymes and other redox molecules serve to counterbalance ROS generated in the cell. A number of signaling pathways are involved in coordinating antioxidant responses to ROSelevation. One critical change in signaling pathways is the activation of transcription factors (TFs), and TF-based reporter vectors are often used to monitor the activation of signaling pathways.

Signosis has developed a set of luciferase reporter vectors (NRF2/ARE, EIK-1, HIF, NFkB, P53, HSF, KLF2 and Negative control) to facilitate antioxidant 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 $1-4 \times 10^5$ 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°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°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 $\mu\text{g}/\text{mL}$ Ampicillin and grow overnight at 37°C.
3. Transfer a single colony to 1-2 ml LB medium containing 100 $\mu\text{g}/\text{mL}$ and shake at 37°C overnight.
4. Prepare plasmids and check on gel.