Signosis
Innovative Plate Assay Solutions

Oxidative Stress Cell Array

Catalog Number CS-1006/CS-1007 **(For Research Use Only)**

Introduction

Oxidative stress is caused by an imbalance in the generation and removal of reactive oxygen species (ROS). ROS elicits a wide spectrum of responses, such as cell proliferation, growth arrest, or 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. Oxidative damage has been implicated in the cause of many diseases, such as cancer, Alzheimer's disease, Parkinson's disease, atherosclerosis, and heart failure. A number of signaling pathways are involved in coordinating the response to elevations in ROS, and the signaling pathways can be monitored through the measurement of related transcription factors (TFs).

In order to analyze the activation of these pathways simultaneously, Signosis has developed Oxidative Stress Pathway Cell Array. In the array, cells have been transiently transfected with 7 different TF luciferase reporter vectors (NRF2/ARE, HIF, NFkB, P53, HSF, AP1, Stat3) and one negative control vector respectively, and the frozen cells are provided in 8 vials. The 8 vials of cells can be further cultured in 8 X 12 wells plate. With the cell array, you can easily monitor whether the treatments of your interest can activate any of these signaling pathways in a simple experiment.

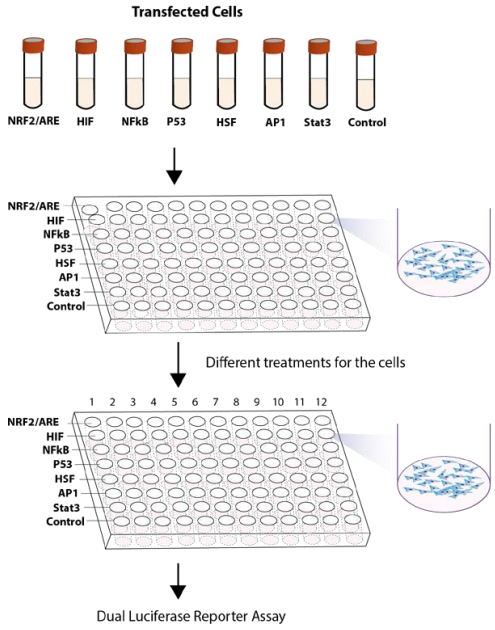

Diagram of Pathway Screening Cell Array I

Principle

In the product line, the cells are pre-transfected with 7 different TF firefly luciferase reporter vectors and one control vector respectively. Renilla luciferase reporter vector is co-transfected with each reporter vector for normalization. Each set of cells consisting 8 vials with different reporters is frozen and stored with stringent criteria to ensure 80-90% cell survival rate. The 8 vials of cells can be arranged and seeded in 8x12 format array in a 96 well plate. The amount of provided cells is enough for 4 (Cat# CS-1006) or 8(CS-1007) x 96 well plates to facilitate the many different experimental conditions, such as duplicate and triplicates. A number of cell lines NIH-3T3, HEK 293, HeLa , Hep G2, Cos-7, are available for your selection.

Materials provided

- 8 vials of 2 X 10^6 cells to 5 X 10^6 cells in Freezing Medium (store the vial in liquid nitrogen until it is ready to be cultured).

Material required but not provided

- Dulbecco's Modified Eagle's Medium (DMEM)
- Fetal Bovine Serum (FBS)
- Penicillin (100 units/ml) Streptomycin (100ug/ml)

Handling cells upon arrival

- It is strongly recommended that you follow instructions as soon as possible upon arrival of cells.

- Prepare **Complete Growth Media**:
  DMEM (in high glucose + sodium pyruvate + L-glutamine + Phenol Red) + Penicillin (100 units/mL) Streptomycin (100ug/ml) + 10% FBS)

Initial Culture Procedure

1. Upon thawing 8 vials of transfected cells, it is recommended to seed the cells right away.
2. Quickly thaw cells in a 37 ^{o}C water bath with constant agitation.
3. Transfer entire contents of the vial to a 1.5ml centrifuge tube and add 10 ml of cold Complete Growth Media to cells.
4. Spin at 3,000 rpm for 5 minutes.
5. Discard supernatant.
6. If you purchase CS-1006, add 10ml of pre-warmed Complete Growth Media to a sterile reservoir. If you purchase CS-1007, add 5ml of pre-warmed Complete Growth Media to a sterile reservoir. (Note: Please use 8 different reservoir for 8 different transfected cells)
7. Transfer 8 resuspended transfected cells to each individual reservoir. Please label clearly as each transfected cells for each individual reservoir.
8. Pipette cells up and down to ensure the transfected cells are mixed well in the medium.
9. Use 100ul of transfected cells for each well.
10. If you purchase CS-1006, use multi-channel pipette to add 100ul of each transfected for each row for a total of 8 X 96-well plate. If your purchase CS-1007, use multi-channel pipette to add 100ul of each transfected for each row for a total of 4 X 96-well plate Rock the culture dish to equally distribute the cells.
    Note: Depending on your experimental design, each 96-well plate can be used for up to 12 treatments or 4 treatments for triplicate setting and 6 samples for duplicate setting.
11. Gently rock the plates to ensure the cells are equally distributed in each well.
12. Place the culture plates with cells in a humidified incubator at 37 ^{o}C with 5% CO2.
13. After overnight culturing, the medium can be replaced with the fresh medium or condition medium. The cells are ready for treatment.

Data Example

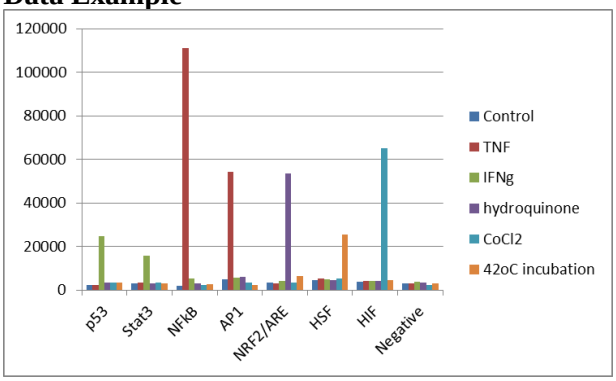

Figure 2. Pathway Screening Cell Array I Analysis. The HeLa cells were cultured in a 96-well plate, and treated without and with TNFa, INFg, Hydroquinone, CoCl2, and 42oC incubation for 8 hours. The luciferase activities were measured with a plate reader.