Signosis, Inc.
Innovative Plate Assay Solutions

DCFDA ROS Kit

Catalog # EA-7001 **(For Research Use Only)**

Introduction

The DCFDA ROS Assay kit utilizes 2',7' dichlorofluorescin diacetate (DCFDA), a cell permeable reagent, to detect reactive oxygen species (ROS) in live cells. DCFDA is capable of measuring hydroxyl, peroxyl, and other ROS activity within the cell. When DCFDA enters the cell, it is deacetylated by cellular esterases into a non-fluorescent compound. Then, it is oxidized by ROS into 2', 7' dichlorofluorescein (DCF), which is highly fluorescent and can be detected by fluorescence spectroscopy at excitation and emission wavelengths of 485 nm/535 nm.

Materials Required but Not Provided

- Cell culture incubator at 37°C
- 96-well black microplate with clear bottom for measuring cell fluorescence
- Microplate reader capable of measuring fluorescence at 485nm/535nm

Materials Provided

- KRPG buffer (RT)
- 10mM DCFDA Reagent (-20°C)

****Spin down small tubes before starting experiment. ****

ROS Measurement

Adherent Cells

1. Seed cells the day before the experiment in a clear bottom 96-well black microplate at 20,000 – 50,000 cells per well, depending on cell type.
2. Incubate the cells overnight in a cell culture incubator at 37°C
3. Prepare a 10 µM DCFDA solution by diluting the 10 mM DCFDA reagent 1:1000 in KRPG buffer. Prepare enough 10 µM DCFDA solution for 100 µL per well used.
4. Optional: for toxicity assays, dilute compound(s) of interest in KRPG buffer to the desired concentration for the experiment (100 µL per well).
5. Carefully remove the media from the wells and wash the cells once with 100 µL of KRPG buffer.
6. Add 100 µL of the 10 µM DCFDA solution to each well.
7. Incubate the cells in a cell culture incubator at 37°C for 45 minutes to stain the cells.
8. Remove the DCFDA solution from each well and add 100 µL of KRPG buffer to each well.
9. Optional: Remove the KRPG buffer from each well and add 100 µL of the diluted compound(s) to each well. Incubate the cells in a cell culture incubator at 37°C for the desired time (1-4 hours).
10. Measure the fluorescence of the plate without removing compounds or buffer in a fluorescence plate reader at Ex/Em = 485/535 nm.

Suspension Cells

1. Grow suspension cells so that approximately 150,000 cells per well are available.
2. Collect cells in a conical tube and wash by centrifugation once in KRPG buffer.
3. Prepare a 10 µM DCFDA solution by diluting the 10 mM DCFDA reagent 1:1000 in KRPG buffer. Prepare enough 10 µM DCFDA solution for 100 µL per well used.
4. Stain the cells by resuspending in 10 µM DCFDA solution. at a concentration of 1,000,000 cells/mL and incubate in a cell culture incubator at 37°C for 30 minutes.
5. Wash cells by centrifugation with KRPG buffer.
6. Resuspend cells in KRPG buffer at concentration of 1,000,000 cells/mL.
7. Seed a clear bottom 96-well black microplate with 100,000 stained cells/well.

8. Optional: Remove the KRPG buffer from each well and add 100 µL of the diluted compound(s) to each well. Incubate the cells in a cell culture incubator at 37°C for the desired time (1-4 hours).
9. Measure the fluorescence of the plate without removing compounds or buffer in a fluorescence plate reader at Ex/Em = 485/535 nm.