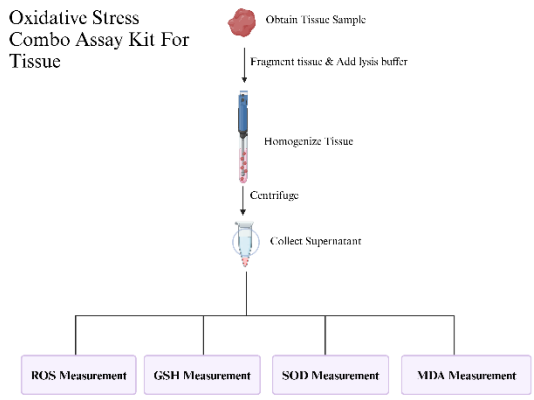
Signosis, Inc.
Innovative Plate Assay Solutions

Oxidative Stress Combo Assay Tissue Kit

Catalog # EA-7008 **(For Research Use Only)**

Introduction

The Signosis **Oxidative Stress Combo Assay Tissue Kit** provides a comprehensive solution for measuring key oxidative stress and antioxidant markers in lysed tissue samples. This kit enables the detection of **reactive oxygen species (ROS), lipid peroxidation (MDA), glutathione (GSH),** and **superoxide dismutase (SOD) activity** by integrating multiple assays into a single kit.



DCFDA Assay

The DCFDA (H2DCFDA) 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. While DCFDA is typically used for cell cultures, it can also be adapted to analyze ROS in tissue samples.

MDA Assay

The Lipid Peroxidation MDA Assay utilizes thiobarbituric acid (TBA) to detect malondialdehyde (MDA) in biological samples. MDA is a marker for oxidative stress and forms from the lipid peroxidation of polyunsaturated fatty acids. When TBA reacts with MDA, a fluorescent red product is formed that can be measured spectrophotometrically at an absorbance of 532 nM.

GSH Assay

The Glutathione (GSH) Assay utilizes Ellman's reagent (DTNB) to measure GSH in biological samples. DTNB reacts with GSH to form a yellow product that can be measured spectrophotometrically at an absorbance of 412 nM.

SOD Assay

The Superoxide Dismutase (SOD) Activity Assay utilizes WST-1 to assess SOD activity in biological samples. SOD is an enzyme that catalyzes the dismutation of the superoxide (O2-) anion radical into molecular oxygen (O2) and hydrogen peroxide (H2O2). This assay indirectly measures the activity of SOD by using WST-8 to detect superoxide O2- levels in samples, which is suppressed when SOD activity is high. WST-8 interacts with superoxide O2- to form a colored product which can be measured spectrophotometrically at an absorbance of 450 nM.

Materials Required but Not Provided

- PBS
- Microplate reader for fluorescence and absorbance detection
- 96-well black microplate with clear bottom for measuring fluorescence
- 96-well clear microplate

Materials Provided

- 10 mM DCFDA Reagent (-20°C)
- 5x Lysis Buffer (4°C)
- 1 mM MDA Stock Solution (-20°C)
- TBA Solution (RT)
- 10 mM GSH Stock Solution (-20°C)
- DTNB Detection Reagent (-20°C)
- WST Reagent (-80°C)
- Substrate Reagent (-20°C)
- 5mM DTPA (-20°C)
- Oxidase Reagent (4°C)

Data Analysis

Conditions	Interpretation
High ROS, High MDA, Low GSH	Oxidative stress damage
High ROS, High SOD, High GSH	Active antioxidant response
Low SOD, Low GSH, High MDA	Severe oxidative damage, impaired defenses

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

Tissue Sample Preparation

1. Dilute 5x Lysis Buffer to 1x Lysis Buffer with ddH_2O.
2. Weigh tissue sample and add 1mL of 1x Lysis Buffer to 100 mg of tissue.
3. Homogenize tissues with grinder on ice.
4. Sonicate lysates on ice.
5. Centrifuge the lysates at 10,000 RPM for 5 minutes to pellet the tissue debris.
6. Collect the supernatant and measure the protein concentration.
7. Dilute the lysates to a concentration of 1 mg/mL.
8. The lysates may be assayed directly or stored away at -80°C.

ROS Measurement

1. Prepare a 10 µM DCFDA solution by diluting the 10 mM DCFDA reagent 1:1000 in PBS. Prepare enough 10 µM DCFDA solution for 90 µL per sample.
2. Prepare samples in each well of a 96-well black plate by diluting 10 µL of the tissue lysates in 90 µL of the 10 µM DCFDA solution.
3. Incubate the plate at 37°C for 30 minutes.
4. Measure the fluorescence of the plate in a fluorescence plate reader at Ex/Em = 485/535 nm.

MDA Measurement

1. Using the provided 1 mM MDA stock solution, prepare a standard curve dilution in a 96-well clear plate as described in the table below:

MDA Dilution Table

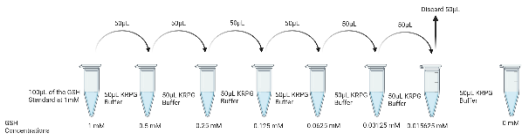
Standard #	1	2	3	4	5	6
MDA Stock Solution Volume (µL)	5	4	3	2	1	0
ddH_2O (µL)	45	46	47	48	49	50
MDA Final Concentration (µM)	100	80	60	40	20	0
Standard Final Volume (µL)	50	50	50	50	50	50

Note: **Ensure to include a blank well as a negative control. **

2. Prepare samples in each well of the plate by diluting 10 µL of the tissue lysates in 40 µL of ddH_2O.
3. Add 50 µL of the TBA solution to each sample or standard in the 96-well plate.
4. Incubate the plate at 95°C for 1 hour. (TBA reactions can be heated in PCR tubes in a thermocycler if desired.)
5. After incubation, cool the plate on ice or a 4°C fridge for 10 minutes.
6. Measure the absorbance of the plate at 532 nM using a plate reader.

GSH Measurement

1. Prepare a GSH standard curve in a 96-well clear plate using an 8-well serial dilution. In the first well, dilute 10 µL of the 10 mM GSH stock solution in 90 µL of PBS to make a 1 mM GSH standard. Next, add 50 µL of PBS to the next 7 wells. Then, transfer 50 µL of the first well to the next well to make a two-fold dilution. Perform six additional two-fold serial dilutions and leave the last, 8th well untouched as the blank buffer well.



2. Prepare samples in each well of the plate by diluting 10 µL of the tissue lysates in 40 µL of PBS.
3. Prepare GSH detection solution by diluting DTNB detection reagent 1:20 in PBS.
4. Add 50 µL of the GSH detection solution to each sample or standard in the 96-well plate.
5. Incubate the plate at 37°C for 10 minutes.
6. Measure the absorbance of the plate at 412 nm using a plate reader. Alternatively, measure the fluorescence of the plate in a fluorescence plate reader at Ex/Em = 340/460 nm.

SOD Measurement

1. Begin preparation of 10 mL of WST working solution by diluting 200 µL WST reagent, 100 µL Substrate reagent, and 100 µL 5mM DTPA in 9.6 mL of PBS.
2. Right before loading the samples, complete the WST working solution by adding 10 µL of the Oxidase reagent to the WST working solution and mixing. **Make sure the Oxidase reagent is evenly resuspended by pipetting up and down before using.**
3. In a clear 96-well plate, add 100 µL of the WST working solution to each well. Add 10 µL of cell sample to each well with WST working solution and mix thoroughly. **Be sure to load the samples quickly, since the WST reaction is active when the Oxidase reagent is added. Use a multichannel pipette if possible.**
4. For the control well, add 10 µL of PBS to one of the wells with WST working solution.
5. Incubate the plate at 37°C for 45 minutes. The plate can be incubated for an additional hour or two if a stronger signal is desired.
6. Measure the absorbance of the plate at 450 nm using a plate reader.