



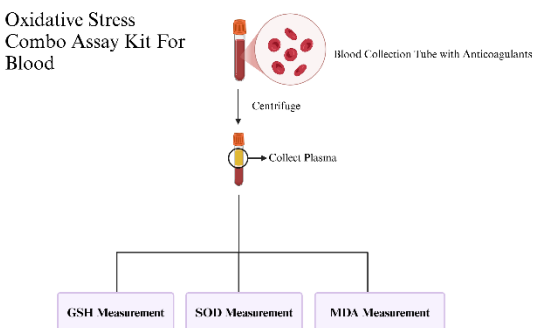
Oxidative Stress Combo Assay Blood Kit

Catalog # EA-7007

(For Research Use Only)

Introduction

The Signosis **Oxidative Stress Combo Assay Blood Kit** provides a comprehensive solution for measuring key oxidative stress and antioxidant markers in blood samples, or other biological fluids. This kit enables the detection of **lipid peroxidation (MDA)**, **glutathione (GSH)**, and **superoxide dismutase (SOD) activity** by integrating multiple assays into a single kit.



Principle

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-8 to assess SOD activity in biological samples. SOD is an enzyme that catalyzes the dismutation of the superoxide (O_2^-) anion radical into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2). This assay indirectly measures the activity of SOD by using WST-8 to detect superoxide O_2^- levels in samples, which is suppressed when SOD activity is high. WST-8 interacts with superoxide O_2^- to form a colored product which can be measured spectrophotometrically at an absorbance of 450 nM.

Materials Required but Not Provided

- PBS
- Cell culture incubator at 37°C
- Microplate reader for fluorescence and absorbance detection
- 96-well clear microplate

Materials Provided

- 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 MDA, Low GSH	Oxidative stress damage
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. ****

Plasma Sample Preparation

1. Centrifuge citrated or EDTA-collected blood at 4°C (1,000 x g for 10 minutes) to separate plasma from erythrocytes. Alternatively, blood collected without anticoagulant can be centrifuged to collect serum.
2. Transfer the plasma layer to a new tube without disturbing the buffy layer.
3. The plasma may be assayed directly or stored away at -80°C.

MDA Measurement

1. Using the provided 1 mM MDA stock solution, prepare a standard curve dilution in PCR tubes as described in the table below:

MDA Dilution Table

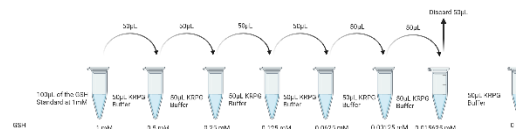
Standard #	1	2	3	4	5	6
MDA Stock Solution Volume (μL)	5	4	3	2	1	0
ddH ₂ O (μL)	45	46	47	48	49	50
MDA Final Concentration (μM)	100	80	60	40	20	0
Standard Final Volume (μL)	50	50	50	50	50	50

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

2. Add 50 μL of plasma or serum samples to PCR tubes. Samples can be diluted in ddH₂O before loading.
3. Add 50 μL of the TBA solution to each sample or standard in the PCR tubes.
4. Heat the PCR tubes in a thermal cycler at 95°C for 1 hour.
5. After heating, cool the PCR tubes on ice or in a 4°C fridge for 10 minutes.
6. Transfer the heated samples from the PCR tubes to 1.5mL centrifuge tubes and centrifuge at >10,000RPM for 1 minute to pellet any precipitate. (The standards do not contain any precipitate and do not need to be centrifuged.)
7. Transfer 60 μL of the standards and the liquid supernatant from the samples without disturbing the pellet to a 96-well clear plate.
8. 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. Add 50 μL of plasma or serum samples to each well of the plate. Samples can be diluted in PBS before loading.
3. Prepare GSH detection solution by diluting DTNB detection reagent 1:20 in PBS buffer.
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. Prepare 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, add 10 μL of the Oxidase reagent to the WST working solution. **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 plasma or serum 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.