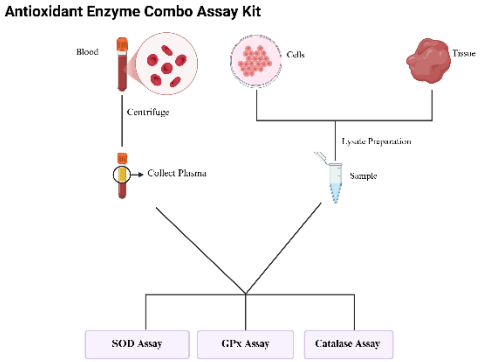
Signosis, Inc.
Innovative Plate Assay Solutions

Antioxidant Enzyme Combo Assay Kit

Catalog # EA-7025 **(For Research Use Only)**

Introduction

Signosis' Antioxidant Enzyme Combo Kit provides an efficient solution for measuring key antioxidant enzymes in biological samples. This kit enables the detection of enzyme activity for superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase by integrating multiple assays into a single kit.



Principle

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 (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.

GPx Assay

The Glutathione Peroxidase (GPx) Activity Assay determines the activity of GPx by measuring its ability to reduce hydrogen peroxide. This assay introduces the enzyme sample into a hydrogen peroxide/glutathione solution and measures how effectively it can remove the hydrogen peroxide from solution. The GPx activity in a sample is quantified by detecting the hydrogen peroxide remaining in solution using a fluorogenic probe that can be measured with a spectrophotometer.

Catalase Assay

The Catalase Activity Assay determines the activity of catalase by measuring its ability to reduce hydrogen peroxide. This assay introduces the enzyme sample into a hydrogen peroxide solution and measures how effectively it can remove the hydrogen peroxide from solution. The catalase activity in a sample is quantified by detecting the hydrogen peroxide remaining in solution using a fluorogenic probe that can be measured with a spectrophotometer.

Materials Required but Not Provided

- PBS
- 96-well clear microplate for absorbance reading or 96-well black microplate with clear bottom for fluorescence reading
- Microplate reader capable of measuring absorbance at 450nm and 560 nm or fluorescence at 530nm/590nm

Materials Provided

- WST Reagent (-80°C)
- Substrate Reagent (-20°C)
- 5mM DTPA (-20°C)
- Oxidase Reagent (4°C)
- Reaction Reagent (-80°C)
- Probe Reagent (-20°C)
- HRP Reagent (4°C)

****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.

Cell Sample Preparation

1. Wash the cells once with PBS before lysing the cells.
2. For a 96-well culture plate, add 40 µL of Lysis buffer to each well and incubate at room temperature for 10 minutes.
3. Pipette the Lysis buffer up and down to detach the cells and transfer the cell lysates into a new tube.
4. If necessary, homogenize the cell lysates with a sonicator.
5. The cell lysates may be assayed directly or stored at -80°C.

Tissue Sample Preparation

1. Weigh tissue sample and add 1 mL of Tissue Lysis buffer per 100mg of tissue.
2. Homogenize the tissue samples with a tissue grinder.
3. If necessary, further homogenize the tissue samples with a sonicator.
4. Centrifuge the sample at 10,000 RPM for 5 minutes to pellet the tissue debris.
5. Collect the supernatant and measure the protein concentration of the supernatant. The tissue sample can be assayed directly or stored at -80°C.
6. Use the Dilution buffer to dilute the tissue sample to the appropriate concentration for each assay.

SOD Activity Measurement

1. Begin preparation of 10 mL of WST working solution by diluting 200 µL WST reagent, 100 µL Substrate reagent, and 100 µL 10mM 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.

GPx Activity Measurement

1. Add 40 µL of Reaction Reagent to each well.
2. Add 40 µL of sample to each well with Reaction Reagent and mix thoroughly.
3. Incubate the plate at room temperature for 30 minutes.

4. Detection mix preparation: calculate the amount of each reagent needed to make the detection mix according to the table below.

Component	Detection Mix (per well/sample)
Probe Reagent	0.8 µL
HRP Reagent	1.6 µL
PBS	48.5 µL
Total	77.6 µL

5. Add 80 µL of detection mix to each reaction well in the plate. **Be sure to add the detection mix quickly, since the signal begins to develop when the reagents are added. Use a multichannel pipette if possible.**
6. Cover the plate and incubate at room temperature away from light for 10-20 minutes.
7. **Exposure to light will produce background signal in wells.**
8. Measure the absorbance of the plate at 560 nm using a plate reader. Alternatively, measure the fluorescence of the plate in a fluorescence plate reader Ex/Em 530nm/590nm.

Catalase Measurement

1. Add 40 µL of Reaction Reagent to each well.
2. Add 40 µL of sample to each well with Reaction Reagent and mix thoroughly.
3. Incubate the plate at room temperature for 30 minutes.

4. Detection mix preparation: calculate the amount of each reagent needed to make the detection mix according to the table below.

Component	Detection Mix (per well/sample)
Probe Reagent	0.8 µL
HRP Reagent	1.6 µL
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Total	77.6 µL

5. Add 80 µL of detection mix to each reaction well in the plate. **Be sure to add the detection mix quickly, since the signal begins to develop when the reagents are added. Use a multichannel pipette if possible.**
6. Cover the plate and incubate at room temperature away from light for 10-20 minutes.
7. **Exposure to light will produce background signal in wells.**
8. Measure the absorbance of the plate at 560 nm using a plate reader. Alternatively, measure the fluorescence of the plate in a fluorescence plate reader Ex/Em 530nm/590nm.