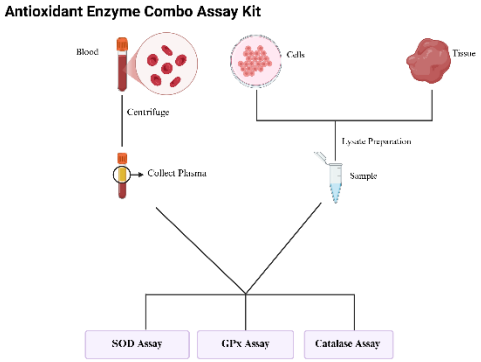
Signosis, Inc.
Innovative Plate Assay Solutions

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 (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.

GPx Assay

The Glutathione Peroxidase (GPx) Activity Assay determines the activity of GPx by measuring its ability to convert reduced glutathione (GSH) to oxidized glutathione (GSSG) in the presence of H2O2. The GSSG that is produced by GPx is quantified using the enzyme glutathione reductase (GR) which interacts with GSSG. GR reduces GSSG to GSH with NADPH as a cofactor, which converts the NADPH to its oxidized form, NADP+. Because NADPH can be measured spectrophotometrically at an absorbance of 340 nm, the depletion of NADPH levels in samples can be used to determine GPx activity. Since GPx activity causes NADPH to be consumed, elevated GPx activity is observed as a decrease in absorbance at 340 nm.

Catalase Assay

The Catalase Activity Assay determines the activity of catalase by measuring its ability to reduce hydrogen peroxide. Catalase is an enzyme that protects cells from oxidative damage by decomposing hydrogen peroxide into water and oxygen. 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, 340 nm, 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)
- 1mM Peroxide Reagent (4°C)
- Serum Reagent (4°C)
- 10mM FAD (-80°C)
- 10mM GSH (-20°C)
- 10mM NADPH (-20°C)
- 1x GR Enzyme Stock (-80°C)
- 100mM Peroxide Reagent (4°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.
6. Use PBS to dilute the cell sample to the appropriate concentration for each assay, if necessary.

Tissue Sample Preparation

1. Weigh tissue sample and add 1 mL of 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 PBS to dilute the tissue sample to the appropriate concentration for each assay, if necessary.

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.

GPx Measurement

1. Sample preparation: A baseline GSSG measurement should be performed additionally for each sample. To obtain a baseline sample, take an aliquot from each sample and heat at 98°C to deactivate all enzymes in the sample. The heated sample contains only the endogenous GSSG from the sample, which will be used as the reference for the baseline GSSG level. The unheated sample will include additional GSSG produced by GPx activity and will have higher overall GSSG levels compared to the baseline.
2. Reaction mix preparation: calculate the amount of each reagent needed to make the reaction mix according to the table below.

Component	Reaction Mix (per well/sample)
1mM Peroxide Reagent	0.9 µL
Serum Reagent	0.9 µL
10mM FAD	0.09 µL
10mM GSH	0.9 µL
10mM NADPH	0.9 µL
1x GR Enzyme	0.09 µL
PBS	86.22 µL
Total	90 µL

3. Any unused enzyme stock can be stored at -80°C for future use.
4. Add 90 µL of reaction mix to each well of the plate.
5. Add 10 µL of sample to each well with reaction mix and mix thoroughly.
6. Cover the plate and incubate at room temperature for 10-20 minutes.
7. Measure the absorbance of the plate at 340 nm using a plate reader. Multiple readings of the plate can be done at 5 minute intervals to observe potential kinetic changes in the measurements.

Catalase Measurement

1. Make Reaction mix by diluting the 100mM Peroxide reagent 1:1000 in PBS.
2. Add 45 µL of Reaction mix to each well.
3. Add 5 µL of sample to each well with Reaction mix and mix thoroughly. **Strong samples that reduce the H_2O_2 too quickly can be diluted further with PBS.**
4. Incubate the plate at room temperature for 30 minutes.
5. 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	0.5 µL
HRP Reagent	1 µL
PBS	48.5 µL
Total	50 µL

6. Add 50 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.**
7. Cover the plate and incubate at room temperature away from light for 15-30 minutes.
   Exposure to light will produce background signal in wells.
8. For a stronger signal, the plate can be incubated for another 30-60 minutes away from light.
9. 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.