Signosis, Inc.
Innovative Plate Assay Solutions

Catalase Assay Kit

Catalog # EA-7024 **(For Research Use Only)**

Introduction

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 560 nm or fluorescence at 530nm/590nm

Materials Provided

- 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.

Catalase Measurement

1. Make Reaction mix by diluting the 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.