Product Manual

OxiSelect™ Catalase Activity Assay Kit, Colorimetric

Catalog Number

STA-341 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Oxidative stress is a physiological condition where there is an imbalance between concentrations of reactive oxygen species (ROS) and antioxidants. However, excessive ROS accumulation will lead to cellular injury, such as damage to DNA, proteins, and lipid membranes. The cellular damage caused by ROS has been implicated in the development of many disease states, such as cancer, diabetes, cardiovascular disease, atherosclerosis, and neurodegenerative diseases. Under normal physiological conditions, cellular ROS generation is counterbalanced by the action of cellular antioxidant enzymes and other redox molecules. Because of their potential harmful effects, excessive ROS must be promptly eliminated from the cells by this variety of antioxidant defense mechanisms.

Hydrogen peroxide is an ROS that is a toxic product of normal aerobic metabolism and pathogenic ROS production involving oxidase and superoxide dismutase reactions. Hydrogen peroxide is poisonous to eukaryotic cells and in high doses can initiate oxidation of DNA, lipids, and proteins, which can lead to mutagenesis and cell death. Catalase is an antioxidant enzyme omnipresent in mammalian and non-mammalian cells that destroys hydrogen peroxide by dismutation. Eukaryotic catalases are heme enzymes found in the liver, kidney, and erythrocytes in high concentrations while the lowest concentrations are in the connective tissues. The enzyme is concentrated in the peroxisome subcellular organelles.

Cell Biolabs' OxiSelect™ Catalase Activity Assay is a fast and reliable kit for the direct measurement of catalase activity from cell lysate, plasma, serum, whole blood, and tissue homogenates. Each kit provides sufficient reagents to perform up to 96 assays in a microtiter plate including blanks, catalase standards and unknown samples. Direct spectrophotometric detection of catalase activity with ultraviolet light can cause interference from proteins and other biological components. The assay utilizes visible light (520 nm), which reduces sample interference. The kit is designed for use in single plate microplate readers as well as readers with high-throughput capabilities. Please read this entire manual prior to performing the assay.

Assay Principle

Cell Biolabs' OxiSelect™ Catalase Activity Assay involves two reactions. The first reaction is the catalase induced decomposition of hydrogen peroxide H_2O_2 into water and oxygen. The rate of disintegration of hydrogen peroxide into water and oxygen is proportional to the concentration of catalase (See Reaction 1 in Figure 1). A catalase-containing sample can be incubated in a known amount of hydrogen peroxide. The reaction proceeds for exactly one minute, at which time the catalase is quenched with sodium azide. The remaining hydrogen peroxide in the reaction mixture facilitates the coupling reaction of DHBS and AAP in conjunction with an HRP catalyst (See Reaction 2 in Figure 1). The quinoneimine dye coupling product is measured at 520nm, which correlates to the amount of hydrogen peroxide remaining in the reaction mixture.

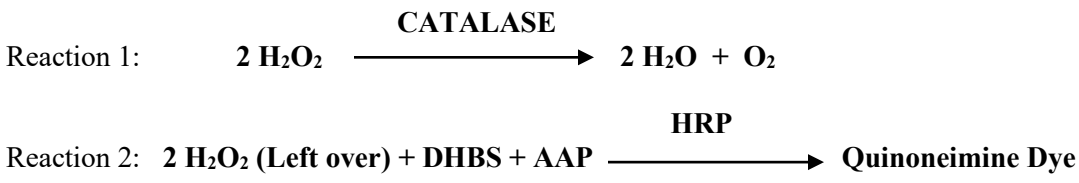

Figure 1: Catalase Assay Principle.

Related Products

1. STA-305: OxiSelect™ Nitrotyrosine ELISA Kit
2. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit
3. STA-312: OxiSelect™ Total Glutathione (GSSG/GSH) Assay Kit
4. STA-330: OxiSelect™ TBARS Assay Kit (MDA Quantitation)
5. STA-340: OxiSelect™ Superoxide Dismutase Activity Assay Kit

Kit Components (shipped at room temperature)

1. Catalase Standard (Part No. 234101): One 50 µL amber tube of 600,000 Units/mL.
2. Hydrogen Peroxide (Part No. 234102): One 100 µL amber tube of an 8.82 M solution.
3. Chromogenic Reagent A (Part No. 234103): One 0.5 mL amber tube.
4. Chromogenic Reagent B (Part No. 234104): One 30 mL bottle.
5. HRP Catalyzer (Part No. 234105): One 50 µL amber tube.
6. Assay Diluent (10X) (Part No. 234106): One 20 mL bottle.
7. Sample Buffer (5X) (Part No. 234107): One 50 mL bottle.
8. Catalase Quencher (Part No. 234108): One 10 mL amber bottle.

Materials Not Supplied

1. Sample extracts for testing
2. Standard 96-well microtiter plates for use in microplate reader
3. Distilled or deionized water
4. Bottles, flasks, and conical or microtubes necessary for reagent preparation
5. Reagents and materials necessary for sample extraction and purification

Warnings and Precautions

- Hydrogen peroxide is corrosive and is harmful by inhalation or if swallowed. Contact with skin may cause burns. In case of contact with eyes, rinse immediately with plenty of water and seek medical attention. Liquid may develop pressure. Keep away from combustible materials.
- Sodium Azide is harmful in contact with skin or if swallowed. Contact with acids liberates a very toxic gas. Azide may react with copper plumbing to form explosive azides. Flush with plenty of water when pouring down a drain.
- Chromogenic Solutions may cause eye or skin irritation. May be harmful if swallowed. May cause respiratory and/or digestive tract irritation.

Storage

Upon receipt, store the kit at 4°C.

Preparation of Reagents

Note: All reagents must be brought to room temperature prior to use.

- 1X Assay Diluent: Dilute the 10X Assay Diluent stock to a 1X solution with distilled water. Mix to homogeneity. Store the 1X Assay Diluent at room temperature.
- 1X Sample Buffer: Dilute the 5X Sample Buffer stock to a 1X solution with distilled water. Mix to homogeneity. Use this for all sample and standard dilutions. Store the 1X Sample Buffer at room temperature.
- Hydrogen Peroxide Working Solution: Prepare a 12 mM Hydrogen Peroxide Working Solution by diluting the stock 8.82 M H_2O_2 solution in Assay Diluent (eg. Add 5 µL of H_2O_2 stock to 3.67 mL Assay Diluent). Prepare only enough Hydrogen Peroxide Working Solution necessary for immediate applications. This reagent is stable for 2 weeks when stored at 2-8ºC and protected from light.
- Chromogenic Working Solution: Prepare only enough Chromogenic Working Solution necessary for immediate applications. Prepare a Chromogenic Working Solution by diluting Chromogenic Reagent A 1:100 with Chromogenic Reagent B. (Example: Add 0.10 mL of Chromogenic Reagent A to 9.9 mL of Chromogenic Reagent B) Mix thoroughly. Next, add 1 µL of HRP Catalyzer per 1 mL of Chromogenic Working Solution. (Example: Add 10 µL to 10 mL of Chromogenic Working Solution) Mix to homogeneity. This reagent is stable for 2 weeks when stored at 4ºC and protected from light.

Preparation of Samples

Note: Samples should be stored at -70°C prior to performing the assay. Sample should be prepared at the discretion of the user. The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design. Samples should be diluted in Sample Buffer unless noted otherwise. Bovine serum albumin can be added to samples with a protein concentration < 0.050 mg/mL to stabilize the enzyme.

- Whole Blood: Collect whole blood (WB) in an anticoagulant tube and mix by inversion. Freeze down 100 µL of whole blood to lyse. Dilute the sample 1:1000 prior to use and use within 1 hour after making diluted preparations. Store any whole blood sample not being immediately used at -70°C. Samples can be stored short-term at 4°C for 10 days, but must be freeze-thawed to lyse cells.
- Plasma: Collect blood with an anticoagulant such as heparin or citrate and mix by inversion. Centrifuge a minimum of 0.5 mL whole blood at 2500 x g at 4°C for 5 minutes. Collect plasma supernatant without disturbing the white buffy layer. Sample should be immediately used or frozen at -70°C for storage. Using a centrifugal ultrafiltration tube with a 30kDa molecular weight cut-off, tare the filter on an analytical balance. Add 125 µL of plasma to the filter tube. Record the weight. Add 375 µL of Assay Diluent. Centrifuge at 10,000 x g for 30 minutes at room temperature. Discard the filtrate. Reconstitute the retained fluid with Assay Diluent to the original weight recorded. Mix solution thoroughly.
- Erythrocyte Lysate: Collect blood in an anticoagulant tube and mix by inversion. Centrifuge a minimum of 0.5 mL whole blood at 2500 x g at 4°C for 5 minutes. Discard the plasma supernatant and wash 5 times in cold 0.9% NaCl. Resuspend the erythrocyte pellet with 4 x the cell-packed volume with ice-cold deionized water. Incubate on ice for 10 minutes. Dilute the sample 1:400 before use. Sample should be tested within 1 hour after making diluted

preparations. Store any unused lysate at -70°C for up to one month. Samples can be stored short-term at 4°C for 5 days.

- Blood Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or stored at -70°C for up to one month. Using a centrifugal ultrafiltration tube with a 30kDa molecular weight cut-off, tare the filter on an analytical balance. Add 125 µL of serum to the filter tube. Record the weight. Add 375 µL of Assay Diluent. Centrifuge at 10,000 x g for 30 minutes at room temperature. Discard the filtrate. Reconstitute the retained fluid with Assay Diluent to the original weight recorded. Mix solution thoroughly.
- Tissue Homogenate: Prior to dissection, perfuse tissue or rinse with a phosphate buffered saline (PBS) solution. This is to remove any red blood cells and clots. Weigh and homogenize the tissue on ice in 5-10 mL cold PBS with 1mM EDTA per gram of tissue. Centrifuge at 10,000 x g for 15 minutes at 4°C. Remove the supernatant and store on ice. Store any unused supernatant at -70°C for up to one month.
- Cell Lysate: Collect cells by centrifuging at 2000 x g for 10 minutes at 4°C. For adherent cells, do not use proteolytic enzymes to harvest cells, but rather use a rubber policeman. Sonicate or homogenize the cell pellet on ice in 1-2 mL cold PBS, 1mM EDTA. Centrifuge at 10,000 x g for 15 minutes at 4°C. Remove the supernatant and store on ice. Aliquot and store the supernatant for use in the assay. Store any unused supernatant at -70°C for up to one month.

Preparation of Catalase Standard Curve

1. Use only enough Catalase Standard as necessary for immediate applications. Immediately prior to use, vigorously vortex the Catalase Standard stock vial to obtain a homogenous suspension and immediately remove the desired amount. It is recommended to pipette up and down several times prior to removing the suspension. Prepare fresh standards by diluting the 600,000 Units/mL Catalase Standard stock 1:60 in Sample Buffer for a 10,000 Units/mL solution. (Example: Add 5 µL of Catalase Standard stock tube to 295 µL of Sample Buffer)
   Note: One unit of catalase is the amount of enzyme that will decompose 1.0 µmole of H_2O_2 per minute at 25°C.
2. Use the 10,000 Units/mL solution to prepare a series of catalase standards according to Table 1 below.
   Note: If crystals are visible upon preparation, gently warm the standards at 30ºC with vortexing.

Tubes	10,000 Units/mL Catalase Standard Dilution (µL)	Sample Buffer (µL)	Catalase Concentration (Units/mL)
1	10	990	100
2	500 of Tube #1	500	50
3	500 of Tube #2	500	25
4	500 of Tube #3	500	12.5
5	500 of Tube #4	500	6.25
6	500 of Tube #5	500	3.125
7	500 of Tube #6	500	1.5625
8	0	500	0

Table 1. Preparation of Standards

Note: Catalase is unstable at high dilutions and should be kept on ice while being used. Use diluted standards within 60 minutes of preparation. Do not store diluted Catalase Standard solutions.

Assay Protocol

Note: Each catalase standard and samples should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add 20 µL of the diluted catalase standards or unknown samples to a 96-well microtiter plate.
2. Add 50 µL of the Hydrogen Peroxide Working Solution (12mM) to each well. Mix thoroughly and incubate at room temperature for exactly 1 minute.
3. Stop the reaction by adding 50 µL of the Catalase Quencher into each well and mix thoroughly.
4. Transfer 5 µL of each reaction well to a fresh well.
5. Add 250 µL of the Chromogenic Working Solution to each well. Incubate the plate at room temperature for 40-60 minutes with vigorous mixing.
6. Read the plate absorbance at 520 nm. Save values for Calculation of Results below.

Example of Results

The following figure demonstrates a typical standard curve for the OxiSelect™ Catalase Activity Assay. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

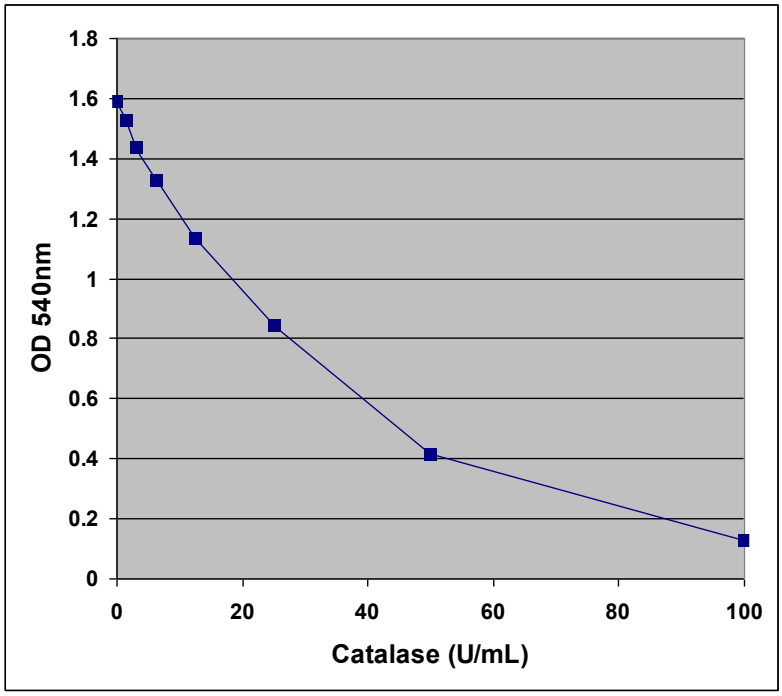

Figure 2: Catalase Activity Assay Standard Curve.

Calculation of Results

The concentration of the unknowns can be determined by interpolation of the catalase standard curve (Figure 2) or analyzed by plate reader software with a 4-parameter logistic curve fitting program to calculate a second order polynomial regression for each sample.

$$A_{520} = ax^2 + bx + c$$

The "A_{520}" is the absorbance of the sample, "x" is the catalase activity in Units/mL, and a, b, and c are the coefficients of the quadratic equation.

To use Microsoft Excel,

1. Plot the catalase standard curve, similar as Figure 3, use OD 520 nm for X axis and Catalase amount as Y axis.
2. Highlight the curve first. Select 'trendline of a second order polynomial and display equation on chart'.
3. Calculate the catalase activity using the chart equation.

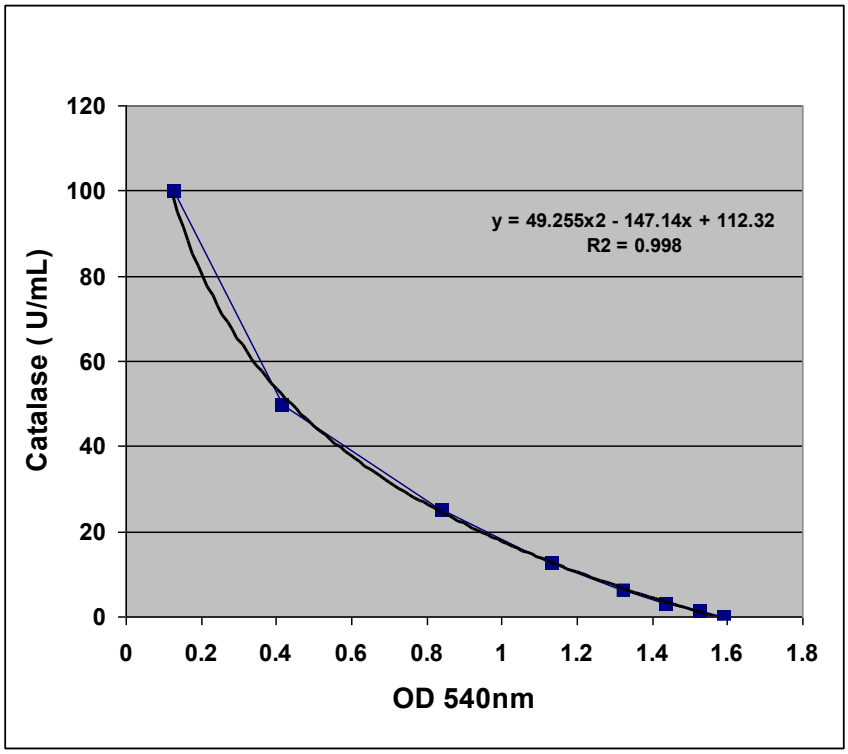

Figure 3: Catalase Activity Assay Standard Curve with second order polynomial equation.

Assay Compatibility

Since blood is one of the sources for catalase, anticoagulants such as heparin, potassium EDTA, or sodium citrate have been tested for compatibility. Various endogenous compounds present in plasma and serum, such as Ascorbic acid and Uric acid, may interfere with the assay. The serum/plasma ascorbic acid and uric acid interference can be minimized by centrifugation with an ultrafiltration device. Whole blood, RBC lysates, or other lysate/tissue sample levels should not cause interference with the results.

Samples are usually diluted significantly before testing; however, tissue samples with low catalase concentrations may lead to a small dilution factor, which can cause endogenous compounds such as hemoglobin or albumin to interfere with the assay. Please see Table 2 below. Use the chart as a guide for preparing samples prior to performing the assay.

Interfering Substance	Compatibility
Ascorbic acid	10-20 µM
Albumin	50 mg/mL
Sodium citrate	20 mM
Tripotassium EDTA	4mM
Hemoglobin	0.75 mg/mL
Heparin	14 Units/mL
Glucose	5 mM
Triton X-100	0.5%

Table 2: Assay Compatibility

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