Catalase (CAT) Activity Colorimetric Assay Kit

Cat#: orb1173251 (User Manual)

Micro Catalase (CAT) Activity Assay Kit
Cat #: orb1173251
Size: 96 T/480 T
Lot #: Refer to product label
Detection range: 2-75 µM **Sensitivity:** 2 µM
Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells
Storage: Stored at -20℃ for 6 months, protected from light

Assay Principle

Catalase (CAT, EC 1.11.1.6) is a common antioxidant enzyme that catalyzes the decomposition of hydrogen peroxide (H2O2) into water and oxygen. It is widely found in aerobic cells containing cytochrome systems. Hydrogen peroxide is highly toxic to cells, and its accumulation leads to oxidation of cellular targets such as DNA, proteins and lipids, leading to mutagenesis and cell death. Protection against oxidative damage to cells is provided by using catalase to remove hydrogen peroxide (H2O2) from cells. The role of catalase in oxidative stress-related diseases has been extensively studied. Catalase also shows peroxidation activity in which low molecular weight alcohols can be used as electron donors. Fatty alcohols are specific substrates for catalase; however, other enzymes with peroxidation activity do not utilize these substrates. CheKine™ Micro Catalase (CAT) Activity Assay Kit provides a simple and easy assay for the study of catalase activity in a variety of biological samples such as serum, plasma, cells and tissue lysates or biological fluids. This assay kit utilizes the peroxidase function of catalase for measuring catalase activity, in the presence of an appropriate concentration of H2O2, catalase reacts with methanol to produce formaldehyde, which can react with chromogens. The absorbance of the product can be measured at 540 nm. The catalase activity in the sample is proportional to the OD value.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	96 T	480 T	
Assay Buffer (10×)	10 mL	50 mL	4℃
Sample Diluent (10×)	10 mL	50 mL	4℃
Lysis Buffer (10×)	10 mL	50 mL	4℃
Methanol	4 mL	20 mL	4℃
Formaldehyde Standard (4.25 M)	100 µL	100 µL	4℃

Catalase (Control)	1	1	-20℃
Potassium Hydroxide	4 mL	20 mL	4℃
Hydrogen Peroxide	1 mL	5 mL	-20℃
Chromogen	4 mL	20 mL	-20℃, protected from light
Potassium Periodate	2 mL	10 mL	4℃

Materials Required but Not Supplied

·Microplate reader or visible spectrophotometer capable of measuring absorbance at OD540 nm
·96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
·Incubator, centrifuge
·Deionized water
·Homogenizer (for tissue samples)

Reagent Preparation

1×Assay Buffer: Dilute Assay Buffer (10×) with deionized water to 1×Assay Buffer. Dilute 2 mL Assay Buffer (10×) concentrate with 18 mL deionized water to 1×Assay Buffer. When stored at 4℃, 1×Assay Buffer is stable for at least two months.
1×Sample Diluent: Dilute Sample Diluent (10×) with deionized water to 1×Sample Diluent. Dilute 5 mL Sample Diluent (10×) concentrate with 45 mL deionized water to 1×Sample Diluent. 1×Sample Diluent should be used to dilute the formaldehyde standards, Catalase (Control), and CAT samples prior to assaying. When stored at 4℃, 1×Sample Diluent is stable for at least two months.
1×Lysis Buffer: Dilute Lysis Buffer (10×) with deionized water to 1×Lysis Buffer. Dilute 5 mL Lysis Buffer (10×) with 45 mL deionized water to 1×Lysis Buffer. When stored at 4℃, 1×Lysis Buffer is stable for at least two months.
Methanol: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Formaldehyde standard: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Diluted Catalase (Control): The vial contains a lyophilized powder of bovine liver CAT and is used as a positive control.
Reconstitute the Catalase (Control) by 0.5 ml 1×Sample Diluent to the vial and Vortex well. Take 10 µL of the reconstituted enzyme and dilute with 4.99 mL 1×Sample Diluent. The diluted enzyme is stable for 30 min at 4℃.
Potassium Hydroxide: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Diluted Hydrogen Peroxide: Dilute 40 µL of Hydrogen Peroxide with 9.96 mL deionized water. The diluted Hydrogen Peroxide solution is stable for 2 h.
Chromogen: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃, protected from light.
Potassium Periodate: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Standard Preparation: Dilute 10 µL Formaldehyde Standard with 9.99 mL of 1×Sample Buffer to obtain a 4.25 mM formaldehyde stock solution. Dilute standards as described in Table (below):

Standard	Formaldehyde (µL)	1×Sample Diluent (µL)	Final Formaldehyde Concentration (µM)
1	4	996	2
2	10	990	5
3	30	970	15
4	60	940	30
5	90	910	45
6	120	880	60
7	150	850	75

Note Final formaldehyde concentration in the 170 µl reaction.

Sample Preparation

1. Animal and Plant Tissues: Weigh 0.1 g tissue, add 1 mL cold 1×Lysis Buffer and homogenize on ice. Centrifuge at 10,000 g for 15 min at 4℃, use supernatant for assay and store on ice.
2. Cells: Collect 5×106 cells into the centrifuge tube, Homogenize the cells on ice in 1 mL cold 1×Lysis Buffer. Centrifuge at 10,000 g for 15 min at 4℃. Use supernatant for assay and store on ice.
3. Serum, Plasma: Serum is collected according to conventional methods, and diluted by 1×Sample Diluent for detection; Blood is collected with anticoagulants and mix upside down. Centrifuge at 600 g for 10 min at 4℃, transfer the supernatant to another new tube and diluted by 1×Sample Diluent for detection.

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for one month. If the protein concentration of the sample is need to determined, it is recommended to use Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 540 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Add the following reagents to the 96-well plate or microglass cuvette:

Reagent	Blank well (μL)	Standard well (μL)	Test well (μL)	Positive well (μL)
1×Assay Buffer	100	100	100	100
Methanol	30	30	30	30
1×Sample Diluent	20	0	0	0
Stds	0	20	0	0
Sample	0	0	20	0
Diluted Catalase (Control)	0	0	0	20
Diluted Hydrogen Peroxide solution	20	20	20	20
Mix well. Incubate for 20 min at room temperature				
Potassium Hydroxide	30	30	30	30
Chromogen	30	30	30	30
Mix well, Incubate for 10 min at room temperature				
Potassium Periodate	10	10	10	10

3. Mix well. Incubate for 5 min at room temperature. Read the absorbance at 540 nm. Finally, calculate ΔATest=ATest-ABlank, ΔA Standard=AStandard-ABlank.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔATest is less than 0.001, increase the sample quantity appropriately. If ΔATest is greater than 0.8, the sample can be appropriately diluted with 1×Sample Diluent, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

1.Drawing of standard curve:
With the final formaldehyde concentration (μM) of the standard solution as the y-axis and the ΔAStandard as the x-axis, draw the standard curve.

2.Calculate the formaldehyde concentration of the samples using the equation obtained from the linear regression of the standard curve substituting corrected absorbance Values for each sample.
Formaldehyde (μM)=y×(0.17 mL÷0.02 mL)**=8.5×y**

3.Calculate the CAT activity of the sample using the following equation. One unit is defined as the amount of enzyme that will cause the formation of 1.0 nmol of formaldehyde per minute at 25°C.
CAT Activity=μM of Sample÷(20 min)×Sample dilution**=0.425×y×Sample dilution (nmol/min/mL)**

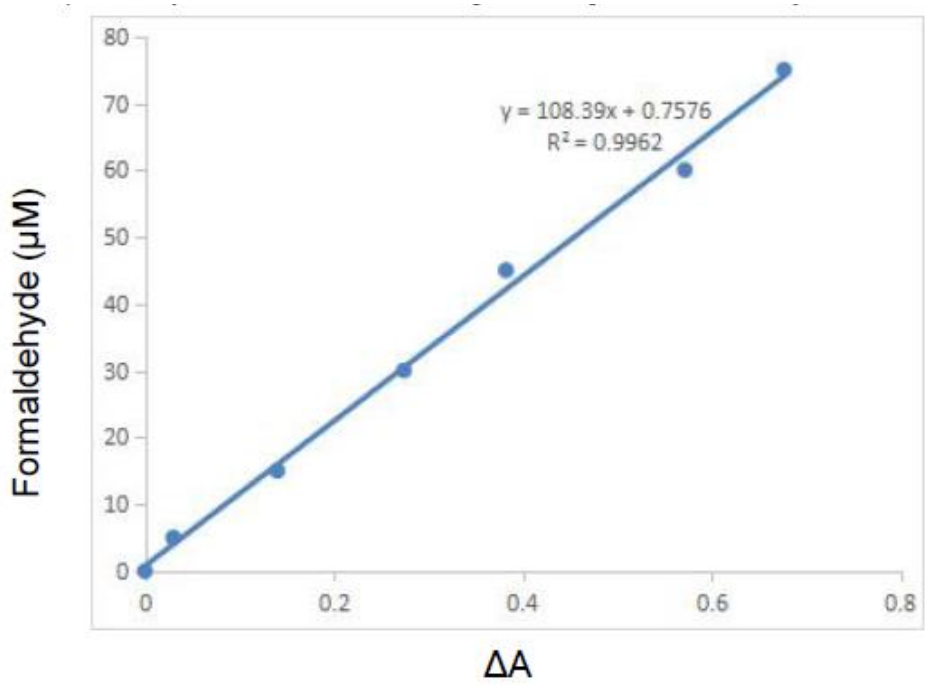

Figure 1. Standard Curve for Catalase