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UC Davis - Catalase

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We use this protocol and it's working

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Abstract

Summary:

Catalase (EC 1.11.1.6; 2H2O2 oxidoreductase) is an ubiquitous antioxidant enzyme that is present in most aerobic cells. Catalase (CAT) is involved in the detoxification of hydrogen peroxide (H2O2), a reactive oxygen species (ROS), which is a toxic product of both normal aerobic metabolism and pathogenic ROS production. This enzyme catalyzes the conversion of two molecules of H2O2 to molecular oxygen and two molecules of water (catalytic activity). CAT also demonstrates peroxidatic activity, in which low molecular weight alcohols can serve as electron donors. While the aliphatic alcohols serve as specific substrates for CAT, other enzymes with peroxidatic activity do not utilize these substrates. In humans, the highest levels of catalase are found in liver, kidney, and erythrocytes, where it is believed to account for the majority of hydrogen peroxide decomposition. The Cayman Chemical Catalase Assay Kit utilizes the peroxidatic function of CAT for determination of enzyme activity. The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H2O2. The formaldehyde produced is measured spectrophotometrically with 4-amino3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) as the chromogen. Purpald specifically forms a bicyclic heterocycle with aldehydes, which upon oxidation changes from colorless to a purple color. The assay can be used to measure CAT activity in plasma, serum, erythrocyte lysates, tissue homogenates, and cell lysates.

Materials

MATERIALS

Assay Kit **Cayman Chemical Company** **Catalog #**707002

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Assay Kit	Cayman	707002
Buffer		
Standard		
Potassium Hydroxide		
Hydrogen Peroxide		
Chromagen		
Potassium Periodate		

Note:
Cayman Chemical RRID:SCR_008945

1 Formaldehyde Standard Wells - Add 100 µl of diluted Assay Buffer, 30µl of methanol, and 20µl of standard (tubes A-G) per well in the designated wells on the plate (see sample plate format, Figure 1, page 12).

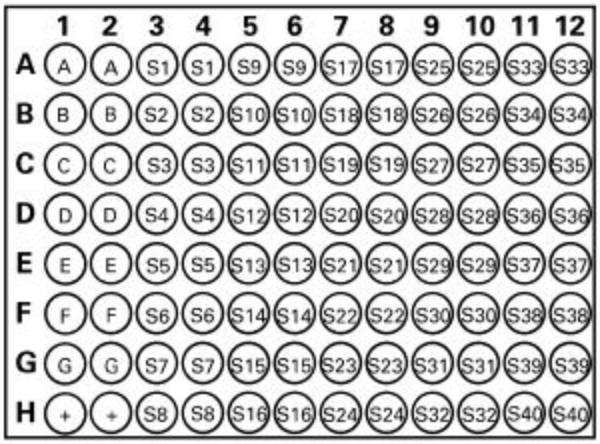

Figure 1. Sample plate format

A - G= Standards
+ = Positive Controls
S1- S40 = Sample Wells

2 Positive Control Wells (bovine liver CAT) - Add 100 µl of diluted Assay Buffer, 30µl of methanol, and 20 µl of diluted Catalase (Control) to two wells.

3 Sample Wells - Add 100 µl of diluted Assay Buffer, 30µl of methanol, and 20 µl of sample to two wells. To obtain reproducible results, the amount of CAT added to the well should result in an activity between 2-35 nmol/min/ml. When necessary, samples should be diluted with diluted Sample Buffer or concertrated with an Amicon centrifuge concentrator with a molecular weight cut-off of 100,000 to bring the enzymatic activity to this level.

4 Initiate the reactions by adding 20 µl of diluted Hydrogen Peroxide to all the wells being used. Make sure to note the precise time the reaction is initiated and add the diluted Hydrogen Peroxide as quickly as possible.

5 Cover the plate with the plate cover and incubate on a shaker for 20 minutes at room temperature.

6 Add 30 µl of diluted Potassium Hydroxide to each well to terminate the reaction and then add 30 µl of Catalase Purpald (Chromagen) (Item No. 707017) to each well.

7 Cover the plate with plate cover and incubate for 10 minutes at room temperature on the shaker.

8 Add 10µl of Catalase Potassium Periodate (Item No. 707018) to each well. Cover with plate cover and incubate five minutes at room temperature on a shaker.

9 Read the absorbance at 540 nm using a plate reader.

10 **Calculation**

1). Calculate the average absorbances of each standard and sample.

2). Subtract the average absorbance of standard A from itself and all other standards and samples.

3). Plot the corrected absorbance of standards (from step 2 above) as a function of final formaldehyde concentration (µM) from Table 1. See Figure 2 for a typical standard curve.

Tube	Formaldehyde (µl)	Sample Buffer (µl)	Final Concentration (µM formaldehyde)*
A	0	1,000	0
B	10	990	5
C	30	970	15
D	60	940	30
E	90	910	45
F	120	880	60
G	150	850	75

Table 1
*Final formaldehyde concentration in the 170 µl reaction.

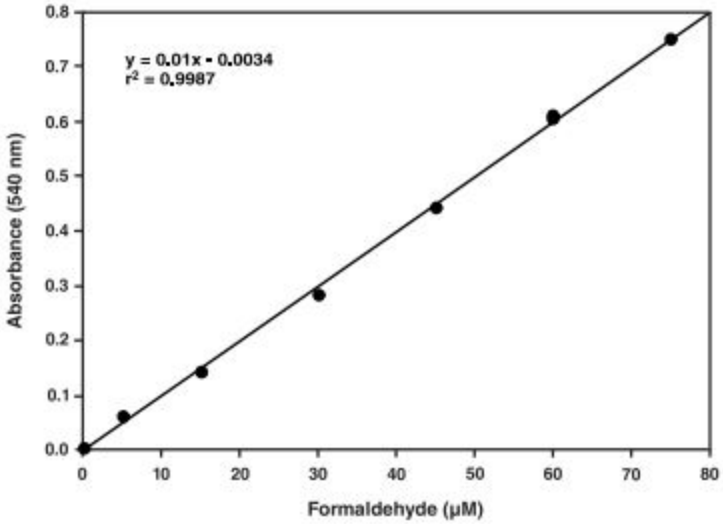

Figure 2. Formaldehyde standard curve

4). 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.

$$\text{Formaldehyde } (\mu M) = \left[\frac{\text{sample absorbance - (y-intercept)}}{\text{slope}}\right] \times \frac{0.17 \text{ ml}}{0.02 \text{ ml}}$$

5). 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 25ºC.

$$\text{CAT Activity} = \frac{\mu M \text{ of sample}}{20 \text{ min.}} \times \text{Sample dilution} = \text{nmol/min/ml}$$