Catalase

Protocol

Version: 1
Replaced by version
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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-amino-3-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.

Reagents and Materials:

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

Protocol:

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

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

3. Sample Wells - Add 100 μl of diluted Assay Buffer, 30 μl 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 concentrated 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 the plate cover and incubate for 10 minutes at room temperature on the shaker.

8. Add 10 μl 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.

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.

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.

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

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 25°C.

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