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Applied Bioanalytical Labs

CAT-240

A Colorimetric Assay Kit for Catalase Activity

(For 30 test tube assays or for 100 microplate assays)

Store at ~4°C

For *In-Vitro* Research Use Only

Introduction

Catalase is an antioxidant enzyme ubiquitously present in aerobic cells. It catalyses the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen. Hydrogen peroxide is formed in cells by controlled pathways. H_2O_2 elicits a broad spectrum of cellular response ranging from mitogenic growth stimulation to apoptosis to necrosis at different concentration levels. Locally intense amount of hydrogen peroxide is produced by inflammatory cells to kill pathogens. Hydrogen peroxide at high concentration is deleterious to cells and its accumulation causes oxidation of cellular targets such as DNA, proteins, and lipids leading to mutagenesis and cell death. Removal of the H_2O_2 from the cell by catalase provides protection against oxidative damage to the cell. The role of catalase in oxidative stress related diseases has been widely known. Catalase activity varies greatly between tissues. The activity is highest in the liver, kidney and erythrocyte, and lowest in connective tissues. In eukaryotic cells the enzyme is concentrated in the subcellular organelles called peroxisomes.

The enzyme consists of 4 subunits of the same size, each of which contains a heme active site to accelerate decomposition of hydrogen peroxide. Catalase exhibits an unusual kinetic behaviour, i.e., it is not possible to saturate the enzyme with substrate H_2O_2 up to 5 M concentration but there is a rapid inactivation of the enzyme above 0.1 M H_2O_2. Therefore, its activity assay is typically carried out at 10 – 50 mM H_2O_2. Because substantially lower concentration than saturated substrate is used, the enzyme activity is dependent on precise concentration of H_2O_2. The most common definition of one catalase unit is the amount of catalase decomposing 1.0 μmoles of hydrogen peroxide per minute at pH 7.0 at 25°C, with initial H_2O_2 concentration of 10.3 mM.

Our kit provides a simple and easy colorimetric rate assay for the study of catalase activity in various biological and purified samples. Our method is essentially that described by Beers and Sizer (1952) in which the decomposition of peroxide is followed spectrophotometrically at 240 nm, with modifications to increase robustness and convenience. Instead of having to calibrate precise H_2O_2 concentration to 10.3 mM in a tedious process, our assay uses a certified catalase standard with known unit activity defined above. Because catalase concentration in sample is obtained by comparing to catalase standards, calibration of precise H_2O_2 concentration is not necessary in our assay. Similarly, experiments can be carried out at room temperature under conditions that are more accurate and convenient. Modifications are also made in our formulations to overcome problems associated with instability of diluted hydrogen peroxide and diluted enzyme standards at the room temperature. There is no need to keep them on ice - wasting time to bring them to the assay temperature before each individual assay.

The assay principal is summarized in the reaction scheme below:

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

(Absorbance at 240 nm↓) (no Absorbance at 240 nm)

The absorbance of hydrogen peroxide at 240 nm is measured directly to calculate the reaction rate since water and oxygen does not absorb at this wavelength. In the presence of catalase, the reaction rate is proportionally (linearly) enhanced.

Reagents

Materials Provided (for 30 test tube test or 100 microplate test):

- Assay Buffer 30 mL
- Hydrogen Peroxide Solution 1 vial
- Sample Dilution Buffer 30 mL
- Catalase Standard (150 U) 1 vial

Items Required but Not Provided:

Spectrophotometer (capable of recording UV absorbance at least every 2 seconds), or microplate reader (capable of recording UV absorbance in kinetic mode)
Disposable semi-micro UV-cuvettes (1.0 mL), or UV microplate
Microcentrifuge tubes
Plastic or glass bottles
Pipettors, adjustable 0.0 – 1.0 mL (8-channel pipettor – 300 µL for microplate assay)
Disposable pipette tips

Storage and Handling

The kit should be stored as a whole in the original box in a refrigerator. Dispose solution after kit's expiration date.

Preparation of solutions

Let kit warm up to room temperature (~ 2 hours when stand still in air, reconstitute Assay Cocktail while waiting). The Hydrogen Peroxide Vial needs to be mixed completely with the Assay Buffer Bottle to form Assay Cocktail Solution. Catalase Standard Vial must be reconstituted with 1.0 mL of Sample Dilution Buffer. The reconstituted Standard Solution should be used within 2 hours at room temperature and is not suitable for longer-term storage. If portions of standards are saved immediately, they can be used again after over night storage at 4^{o}C. Only prepare catalase standards immediately before use. Total assay time (30 tube assays) without interruption is approximately 1.5 hours.

Assay Cocktail:

Transfer the entire content (~0.46 mL) of the Hydrogen Peroxide Vial into the Assay Buffer Bottle, mix. Wash the vial three times with the mixed solution and combine to the Assay Buffer Bottle. Incubate one hour before using. Catalase standards and samples should be assayed using the same cocktail. The assay cocktail is stable for at least 2.5 hours at room temperature after first assay, and can be stored over night at 4^{o}C (warming up to room temperature again before using, standards need to be assayed again along with samples).

Catalase Standard:
Warning: Only reconstitute and make subsequent dilutions immediately before use, the standards are only stable for 2 hours at room temperature. If portions of these standards are saved immediately after they are made and stored at 4°C, they can be used again after over night storage. Don't freeze prepared standards due to enzyme inactivation upon freezing.

Add 1.0 mL of Sample Dilution Buffer to the Catalase Standard vial, the resultant solution is 150 U/mL in catalase activity. Dilute further in additional four microcentrifuge tubes by mixing following:

Standard No.	Sample Dilution Buffer (µL)	150 U/mL Standard (µL)	Standard Conc. (U/mL)
1	400	0	0.0
2	300	100	37.5
3	200	200	75.0
4	100	300	112.5
5	0	400	150.0

Samples:
Catalase seems to be reasonable stable at high concentration. However, relatively rapid decline has been observed in diluted samples. For example, catalase in concentrated hemolysates (~5g Hb/mL) is stable for 6 days at 2°C, but its activity decreases by 10-15% in 24 hours at 1.2 mg Hb/mL. Freezing at –20°C is to be avoided even for concentrated samples. For example, RBC lysate stored at –20°C will lose 40% activity. If it becomes necessary, store the concentrated samples at –70°C or at a lower temperature to avoid activity loss. Our kit formulation stabilizes catalase after dilution up to 2 hours at room temperature. It is important to incubate diluted sample for15 minutes before assaying in our formulation. Diluted sample can be placed on ice and be assayed simultaneously in high-throughput microplate format to ensure accurate results.

Following is approximate guideline of dilution factors (using Sample Dilution Buffer, incubating 15 minutes before assaying):
RBC lysate (1:4 RBC:dH_2O): 1/100x
Tissue Homogenates (5% or 10% with 0.01% digitonin or 0.25% sodium cholate detergent): 1/20x – 1/100x

If the reaction rate exceeds the highest catalase standard rate, it is important not to use the linear regression equation of the standard curve to calculate catalase activity in the sample. Adjust the dilution factor and make a new dilution. It is also possible by adding a smaller volume of the diluted sample and making up the total sample volume required with the Sample Dilution Buffer directly in the assay mixture specified in the Procedures. For example, instead of adding 50.0 µL of a secondary 1/2x dilution of a diluted sample, one may add 25.0 µL of the diluted sample and 25.0 µL of Sample Dilution Buffer).

Procedure

Cuvet Assay

1. Turn on spectrophotometer and let it warm up according to manufacturer's instruction for UV measurement (relatively constant room temperature lab environment is recommended).
2. To a clean cuvette, add 1000.0 µL of Sample Dilution Buffer. Place it in the reference cuvette holder. Set wavelength to 240 nm and zero the instrument (absorbance).
3. To a clean semi-micro UV cuvette, add 950.0 µL of Assay Cocktail. Pipette 50.0 µL of standard or sample to the cuvet, mix as quickly as possible by repeated pippetting (~10 times) with the same pipette tip, or by capping/inverting the cuvet. Immediately start recording absorbance at 240 nm, every 2 second or at the smallest time interval allowed for 0.25 minutes (15 seconds). Keep the same pace of pippetting/mixing throughout the whole experiment (do not allow pippetting/mixing time to exceed 15 seconds, a practice run with a timer using Sample Dilution Buffer or dH_2O is recommended).

Microplate Assay

Because data have to be recorded within ~30 seconds after mixing sample and assay cocktail, the assay can only be performed ***one column (8 wells) or one well at a time.*** *Consult microplate reader manual for the minimum interval of reading time in the kinetic mode, the interval is typically listed for 96-well reading and 1/10 of the interval is needed to read a whole column of wells. About 7 data points are needed to obtain reaction rate. Do not attempt to assay more than one column at the same time.*

1. Turn on microplate reader and let it warm up according to manufacturer's instruction for UV measurement (relatively constant room temperature lab environment is recommended). Set wavelength to 240 nm, and set up microplate layout and other parameters.
2. To each clean UV microplate well, pipette 15.0 µL of standard or sample. Add 290.0 µL of Assay Cocktail, using an 8-channel pipettor when needed, to each well. Mix as quickly as possible by shaking (within microplate programming). Immediately start recording absorbance at 240 nm, every 2 second or at the smallest time interval allowed for 0.25 minutes (15 seconds). Keep the same pace of pipetting/mixing throughout the whole experiment (do not allow pippetting/mixing time to exceed 15 seconds, a practice run with a timer using Sample Dilution Buffer or dH_2O is recommended).

Typical Results and Calculations:

The decomposition rate of hydrogen peroxide follows a first order reaction with H_2O_2 concentration, and is linear for the first half minute (30 seconds) of reaction (see Figure 1A). The decomposition rate increases with the presence of higher concentration of catalase. Without a rapid-mixing device, it is not possible to record data when sample and reaction cocktail are initially mixed. Complete mixing in a cuvet typically requires about 10 seconds with repeated pipetting. Therefore, reaction has been proceeded when data recording started, and only ~15 seconds of data are available to extract the decomposition rate. Typical rate curves of standards ranging from 0 U/mL to 150 U/mL are shown below in Figure 1. The rate

of reaction can be obtained by linear regression. In the regression equation, Y = -aX + b, a ($\Delta A240_{nm}$/min) is the decomposition rate. A less preferred way to obtain the rate is to select two data points in the rate curve and to calculate rate according to following equation: Rate($\Delta A240_{nm}$/min) = -(Y2 – Y1)/(X2 – X1). The decomposition rates thus obtained are used to plot against the concentration of catalase and to construct a standard curve (rate vs catalase concentration, see Figure 1B).

The linearity of standard curve is lost when catalase concentration exceeds 180 U/mL due to rapid consumption of hydrogen peroxide (data not shown). Therefore, if a sample yields a decomposition rate higher than that of the 150 U/mL catalase standard, the sample has to be diluted further and assayed again.

Calculations:

1. Plot standard curve as illustrated in Figure1B. Obtain the slope (a) and interception (b) of the curve by regression:

$$y = ax + b$$

2. The catalase concentration in the samples added to the reaction mixture (not concentration in the reaction mixture) is:

$$C_i = (R_i - b)/a$$

where R_i is the decomposition rate ($\Delta A240_{nm}$/min) of sample i.

3. The catalase concentration in the original sample is

$$C^O_i = C_i * (\text{Dilution Factor}_i)$$

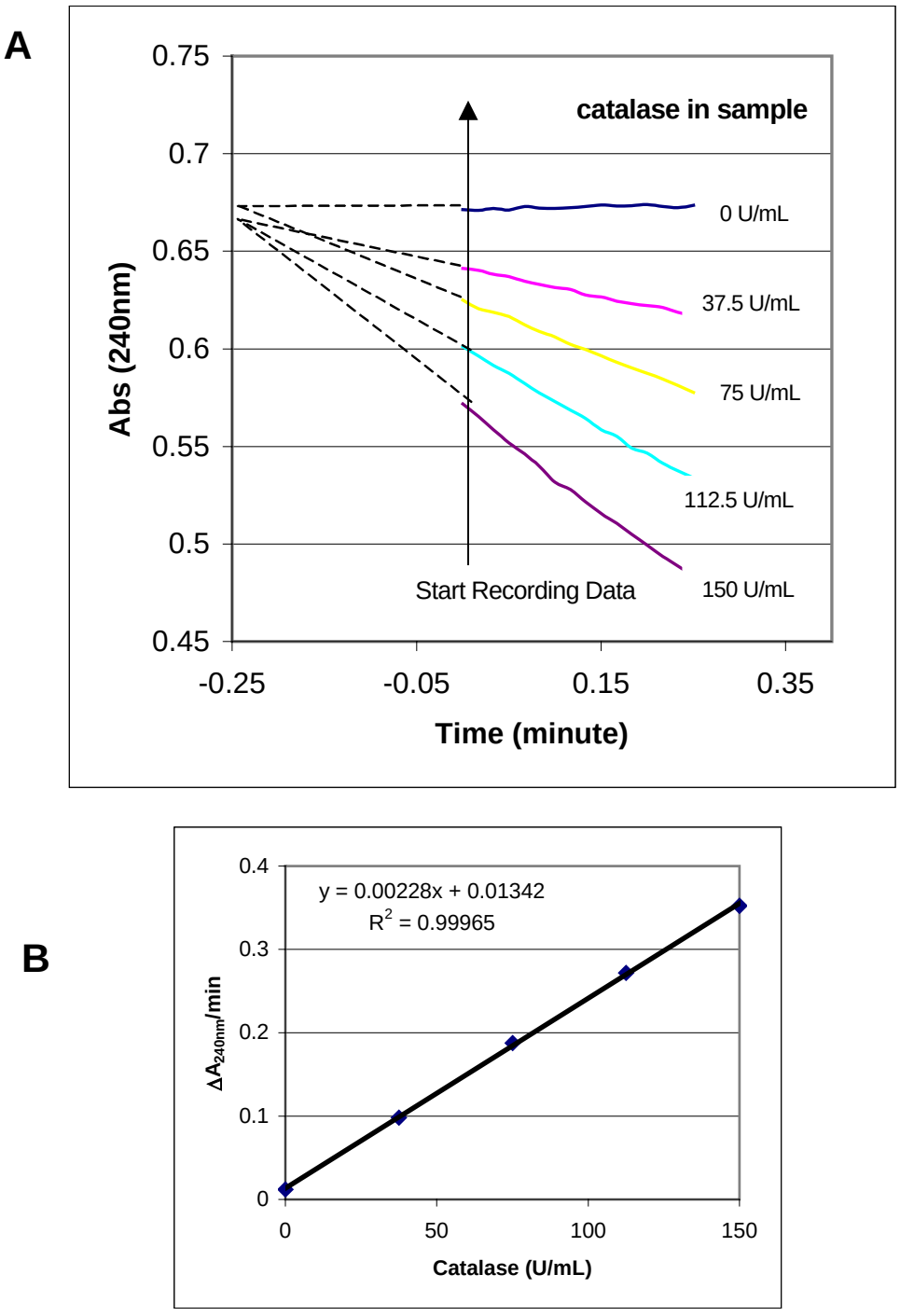

Figure 1: A) Rate curves of hydrogen peroxide decomposition catalyzed by bovine liver catalase. Catalase unit represents concentration in sample added, not in final assay mixture. 50 µL of sample was added to 950 µL of assay cocktail, addition and mixing took about 0.25 minutes. Data recording started at time 0 minute. The experiment was performed at room temperature without a constant temperature curvette holder. B) Standard curve obtained from linear regression rates of hydrogen peroxide decomposition in the presence of catalase.

Specifications (Performance Characteristics for Rate Assay without a Constant Temperature Module):

LLD: The lower limit of detection is 6 U/mL catalase in the samples added to the reaction mixture (0.3 U/mL in the reaction mixture).
Linearity: Linearity is maintained up to 150 U/mL of catalase (in samples).
Assay Precision: The intra-assay coefficient of variation (CV) is 7% for the 37.5 U/mL standard and 5% for the 112.5 U/mL standard.
Recovery: The recovery of the spiked catalase in BRC lysate is 110%.

References

1. Beers, R. F. Jr. and Sizer, I. W., "A Spectrophotometric Method for Measuring the Breakdown of Hydrogen Peroxide by Catalase" , *J. Biol. Chem. 195*, 133-140 (1952).
2. Aebi, H., "Catalase *in Vitro*", Methods in Enzymology 105, 121-126 (1984).

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