CheKine™ Catalase (CAT) Activity Assay Kit

Item NO.	**Product Name**
KTB1040	CheKine™ Catalase (CAT) Activity Assay Kit

ATTENTION

For laboratory research use only. Not for clinical or diagnostic use

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INTRODUCTION

Background

Catalase (EC 1.11.1.6), is a common antioxidant enzyme that catalyzes the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen, ubiquitously present in aerobic cells containing a cytochrome system. Hydrogen peroxide is highly deleterious to the cell and its accumulation will cause oxidation of cellular targets such as DNA, proteins, and lipids, thus leading to mutagenesis and cell death. Removal of the hydrogen peroxide (H_2O_2) from cells by using catalase provides protection against oxidative damage to the cell. The role of catalase in oxidative stress related diseases has been widely studied. Catalase also demonstrates peroxidatic activity, in which low molecular weight alcohols can serve as electron donors. Aliphatic alcohols are specific substrates for catalase, however, other enzymes with peroxidatic activity do not utilize these substrates.

(Catalytic Activity) $2H_2O_2 \xrightarrow{\text{Catalase}} O_2+2H_2O$

(Peroxidatic Activity) $H_2O_2 + AH_2 \xrightarrow{\text{Catalase}} A+2H_2O$

Assay principle

Catalase Activity Assay Kit provides a simple and easy colorimetric assay for the study of catalase activity in a variety of biological samples such as cell and tissue lysates or biological fluids. This assay kit utilizes the peroxidatic function of catalase for measuring catalase activity, based on the reaction of catalase with methanol, with the presence of an optimal concentration of H_2O_2. The formaldehyde produced can be measured colorimetrically at OD 540 nm. Therefore, the catalase activity present in the sample is proportional to the signal obtained.

Storage/Stability

Storage at –20°C and Keep from light immediately upon receipt. Kit has a storage time of 6 months from receipt. Refer to list of materials supplied for storage conditions of individual components.

Assay Restrictions

- Assay kit is intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

PRODUCT INFORMATION

Materials supplied and Storage conditions

Kit components	Quantity			Storage conditions
	48T	96T	480T	
Assay Buffer (10×)	5 mL	10 mL	50 mL	4ºC
Sample Diluent (10×)	5 mL	10 mL	50 mL	4ºC
Formaldehyde standard (4.25 M)	100 µL	100 µL	100 µL	4ºC
Catalase (control)	1 vial/ Lyophilized	1 vial/ Lyophilized	2 vials/ Lyophilized	-20ºC
Potassium Hydroxide	2 mL	4 mL	20 mL	4ºC
Hydrogen Peroxide	0.5 mL	1 mL	5 mL	-20ºC
Chromogen	2 mL	4 mL	20 mL	-20ºC protect from light
Potassium Periodate	1 mL	2 mL	10 mL	4ºC

Other supplies required, Not Supplied

- Standard microplate reader capable of measuring absorbance at 540 nm
- Precision pipettes, disposable pipette tips
- Methanol
- Distilled or deionized water
- Assorted glassware for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom
- Dounce homogenizer (for tissue samples)

Technical hints

- Avoid foaming or bubbles when mixing or reconstituting components.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

ASSAY PROTOCOL

Reagent preparation

1. Assay Buffer (10×): Dilute 2 ml of Assay Buffer concentrate with 18 ml of distilled or deionized water. This final Assay Buffer should be used in the assay. When stored at 4˚C, this diluted Assay Buffer is stable for at least two months.
2. Sample Diluent (10×): Dilute 5 ml of Sample Diluent concentrate with 45 ml of distilled or deionized water. This final Sample Diluent should be used to dilute the formaldehyde standards, Catalase (Control), and CAT samples prior to assaying. When stored at 4˚C, this diluted Sample Diluent is stable for at least two months.
3. Formaldehyde standard: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.
4. 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 diluted Sample Diluent to the vial and Vortex well. Take 10 µl of the reconstituted enzyme and dilute with 4.99 ml of diluted Sample Diluent. A 20 µl of this diluted enzyme per well causes a marked absorbance of approximately 0.3 after subtracting the background absorbance. The diluted enzyme is stable for 30minutes. The reconstituted Catalase (control) is stable for one month at -20°C.
5. Potassium Hydroxide: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.
6. Hydrogen Peroxide: Dilute 40 µl of Hydrogen Peroxide with 9.96 ml of distilled or deionized water. The diluted Hydrogen Peroxide solution is stable for two hours.
7. Chromogen: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.
8. Potassium Periodate: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C

ASSAY PROTOCOL

Sample preparation

Note: If not assayed immediately, samples can be stored at -80°C for one month.

1. Tissue Homogenate: Perfuse tissue with ice-cold PBS to remove red blood cells. Homogenize the tissue on ice at 5 mL/g in cold lysis buffer (50 mM potassium phosphate, pH 7.0, containing 1 mM EDTA). Centrifuge at 10,000g for 15 minutes at 4°C. Use supernatant for assay and store on ice**.**

2. Cell Lysate

1) **Suspension cells:** Centrifuge 1-2 x 10^6 cells at 1,000-2,000 x g for 10 minutes and discard supernatant. Wash cells with cold PBS. Homogenize or sonicate the cell pellet on ice in 1 ml of ice-cold buffer (50 mM potassium phosphate, pH 7.0, containing 1 mM EDTA). Centrifuge at 10,000 x g for 15 minutes at 4°C. Use supernatant for assay and store on ice.

2) **Adherent cells:** Wash 1-2 x 106 cells with cold PBS. Place dish on ice, do not harvest using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate the cell pellet on ice in 1 ml of cold buffer (50 mM potassium phosphate, pH 7.0, containing 1 mM EDTA). Centrifuge at 10,000 x g for 15 minutes at 4°C. Use supernatant for assay and store on ice.

3) **Blood samples:** Collect serum, or plasma (heparin, citrate or EDTA) using standard protocols for assay and store on ice. The erythrocyte pellet can be lysed in 4 x volume of cold dH2O; Centrifuge at 10,000 x g for 15 minutes at 4°C. Collect the supernatant (erythrocyte lysate) for assay and store on ice.

ASSAY PROCEDURE

Standard Preparation

Preparation of the Formaldehyde Standards: Dilute 10 µl of Formaldehyde Standard with 9.99 ml of diluted Sample Buffer to obtain a 4.25 mM formaldehyde stock solution. Dilute standards as described in Table 1 (below).

standard	Formaldehyde (µl)	Sample Diluent (µl)	Final Concentration (µM Formaldehyde)*
1	0	1000	0
2	4	996	2
3	10	990	5
4	30	970	15
5	60	940	30
6	90	910	45
7	120	880	60
8	150	850	75

*Final formaldehyde concentration in the 170 µl reaction.

Performing the Assay

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and control be assayed in duplicate.

1. Formaldehyde Standard Wells: Add 100 µl of diluted Assay Buffer, 30 µl of methanol, and 20 µl of Standard per well.
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) per well.
3. Sample Wells: Add 100 µl of diluted Assay Buffer, 30 µl of methanol, and 20 µl of sample per well. When necessary, samples should be diluted with diluted Sample Diluent.
4. Initiate the reactions by adding 20 µl of diluted Hydrogen Peroxide to all the wells.
5. Cover the plate and incubate on a shaker for 20 minutes at room temperature.
6. Add 30 µl of Potassium Hydroxide to each well and then add 30 µl of Chromogen to each well.
7. Cover the plate and incubate on a shaker for 10minutes at room temperature.
8. Add 10 µl of Potassium Periodate to each well. Cover the plate and incubate on a shaker for 5 minutes at room temperature.
9. Read the absorbance at 540 nm using a plate reader.

DATA ANALYSIS

Calculation of results

1. Calculate the average absorbances of each standard and sample.
2. Subtract the average absorbances of standard 1 form 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.

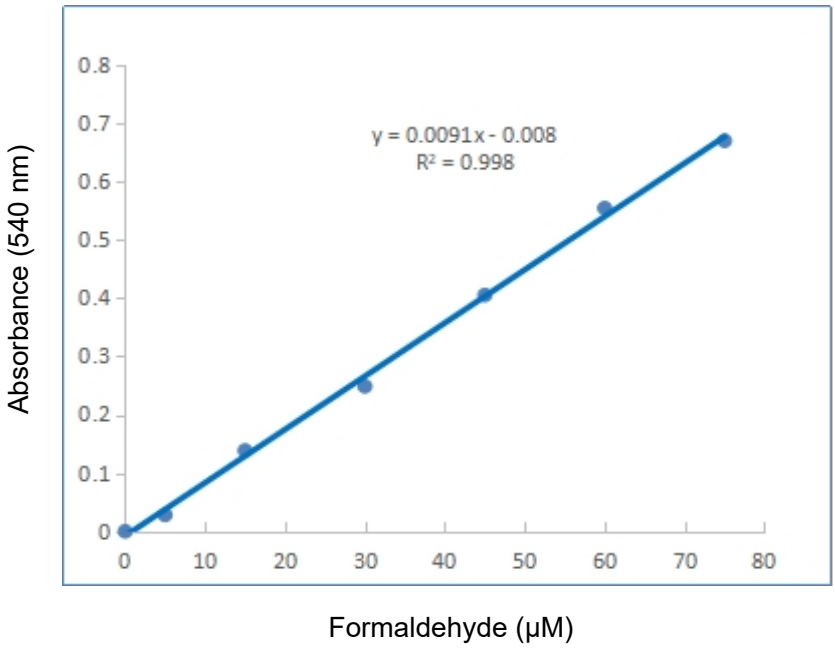

Figure 2. Formaldehyde standard curve

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.

$$\text{Formaldehyde (μM)} = \left[\frac{\text{Sample absorbance} - (\text{y} - \text{intercept})}{\text{Slope}}\right] \times \frac{0.17\ \text{ml}}{0.02\ \text{ml}}$$

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 25°C.

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