Catalase Activity Assay Kit

HTS-ready colorimetric & fluorometric assay for measuring catalase activity in biological samples

PromoKine

Instruction Manual

Catalog Number	PK-CA577-K773
Description	Catalase (EC 1.11.1.6) is a ubiquitous antioxidant enzyme that is present in nearly all living organisms. It functions to catalyze the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen. PromoKine's Catalase Assay Kit provides a highly sensitive, simple, direct and HTS-ready assay for measuring Catalase activity in biological samples. In the assay, catalase first reacts with H_2O_2 to produce water and oxygen, the unconverted H_2O_2 reacts with OxiRed probe to produce a product, which can be measured at 570 nm (Colorimetric method) or at Ex/Em = 535/587 nm (fluorometric method). Catalase activity is reversely proportional to the signal. The kit can detect 1 µU or less of catalase activity in samples.
Quantity	100 assays

Kit Components

Components	Quantity	Color Code
Catalase Assay Buffer	25 ml	NM
OxiRed Probe (in DMSO)	0.2 ml	Red
HRP (lyophilized)	1 vial	Green
H_2O_2 Substrate (0.88 M)	25 µl	Yellow
Stop Solution	1 ml	White
Catalase Positive Control	2 µl	Blue

Storage and Reagents Preparation

Store the kit at -20°C, protect from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge vials prior to opening. Read the entire protocol before performing the assay.

- **OxiRed Probe**: Briefly warm to completely melt the DMSO solution. Store at 4°C, protected from light. Use within two months.
- **HRP**: Dissolve with 220 µl Assay Buffer. Store at 4°C. Use within two months.
- **Positive Control Solution**: Add 500 µl Assay Buffer to Positive Control. Aliquot and store at -20°C. Diluted Positive Control solution is stable for 2-3 days at 4°C, for 2 months at -20°C.

Note: Keep samples, HRP and Catalase on ice while in use.

Assay Protocol

1. Sample and Positive Control Preparations: Homogenize 0.1 gram tissues, or 10^6 Cells, or 0.2 ml Erythrocytes on ice in 0.2 ml cold Assay Buffer; Centrifuge at 10,000 x g for 15 minutes at 4°C; Collect the supernatant for assay, keep on ice. Liquid samples can be tested directly. Store samples at -80°C to assay later.

Add 2 - 78 µl of samples or 1 - 5 µl Positive Control Solution into each well, and adjust volume to total 78 µl with Assay Buffer. Prepare sample High Control (HC) with the same amount of sample in separate wells then bring total volume to 78 µl with Assay Buffer. Add 10 µl of Stop Solution into the sample HC, mix and incubate at 25°C for 5 minutes to completely inhibit the catalase activity in samples as High Control. For unknown samples, we suggest testing several doses of your sample to ensure the readings are within the linear range.

Reducing agents in samples interfere with the assay. Keep DTT or β-ME below 5 µM.

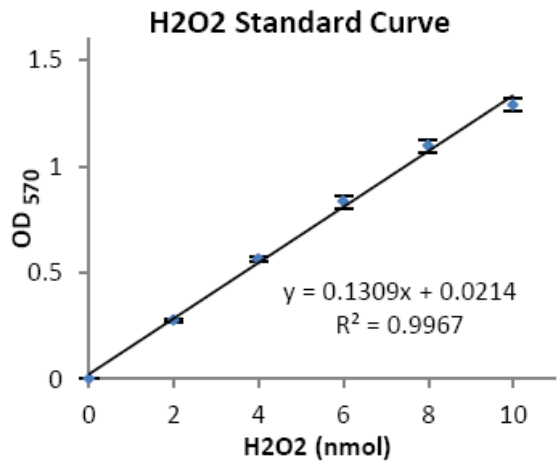
2. H_2O_2 Standard Curve: Dilute 5 µl of 0.88M H_2O_2 into 215 µl dH2O to generate 20 mM H_2O_2, then take 50 µl of the 20 mM H_2O_2 and dilute into 0.95 ml dH_2O to generate 1 mM H_2O_2. Add 0, 2, 4, 6, 8, 10 µl of 1 mM H_2O_2 solution into 96-well plate to generate 0, 2, 4, 6, 8, 10 nmol/well H_2O_2 standard. Bring the final volume to 90 µl with Assay Buffer. Add 10 µl Stop Solution into each well. For the fluorometric assay, dilute the standard H_2O_2 10-fold for the standard curve (0-1 nmol range).

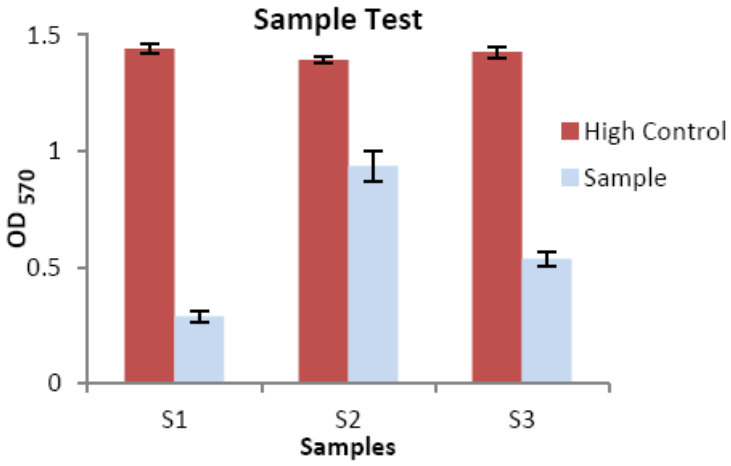
Note: Diluted H_2O_2 is unstable, prepare fresh dilution each time.

3. Catalase Reaction: Add 12 µl fresh 1 mM H_2O_2 into each well (samples, positive control, and sample HC) to start the reaction, incubate at 25°C for 30 minutes, and then add 10 µl Stop Solution into each sample well (Sample, Positive Control; do not add Stop Solution to the HC) to stop the reaction (Note: High Control and standard curve wells already contain Stop Solution).

4. Develop Mix: Mix enough reagents for the number of assays to be performed. For each well prepare a 50 µl Developer Mix containing:

	46 µl Assay Buffer 2 µl OxiRed Probe 2 µl HRP solution Add 50 µl of the Developer Mix to each test samples, controls, and standards. Mix well and incubate at 25°C for 10 minutes. Measure OD 570 nm in a plate reader. Note: For low amounts of catalase, you can either increase the incubation time prior to adding the Stop Solution or use the fluorometric method. For the fluorometric method, decrease the 1 mM H_2O_2 amount to1.5 µl and OxiRed Probe to 0.3 µl in the reaction; compensate the volume with Assay Buffer. **5. Calculation**: Signal change by catalase in sample is $\Delta A = A_{HC} - A_{sample}$. AHC is the reading of sample High Control, A_{Sample} is the reading of sample in 30 minutes. Plot the H_2O_2 Standard Curve. Apply the ΔA to the H_2O_2 standard curve to get B nmol of H_2O_2 decomposed by catalase in 30 minutes reaction. Catalase activity can be calculated: $$\textbf{Catalase Activity} = \frac{B}{30 \times V} \times \textbf{Sample Dilution Factor} = \textbf{nmol/min/ml} = \textbf{mU/mL}$$ Where: B is the decomposed H_2O_2 amount from H_2O_2 Standard Curve (in nmol). V is the pretreated sample volume added into the reaction well (in ml). 30 is the reaction time 30 minutes. Unit definition: One unit of catalase is the amount of catalase that decomposes 1.0 µmol of H_2O_2 per minute at pH 4.5 at 25 °C.
Intended Use	For in vitro research use only. Not for diagnostic or therapeutic procedures.





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