

Catalase Activity Colorimetric/Fluorometric Assay Kit

Cat. No. Kit-0178 Lot. No. (See product label)

SPECIFICATION

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 ₂ O ₂) to water and oxygen. 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 ₂ O ₂ to produce water and oxygen, the unconverted H ₂ O ₂ reacts with OxiRed probe to produce a product, which can be measured at 570 nm (Colorimetric method) or at Ex/Em=535/587nm (fluorometric method). Catalase activity is reversely proportional to the signal. The kit detects high pico-unit of catalase in samples.
Applications	The kit detects high pico-unit of catalase in samples.
Storage	Store kit at 4°C, protect from light. Warm the assay buffer to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.
Shipping	Gel Pack
Size	100 assays
Handling	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 µAssay Buffer. Store at 4°C. Use within two months. Positive Control Solution: Add 500 µl Assay Buffer to Positive Control. Aliquot and

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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.

Kit Components

- Catalase Assay Buffer
- OxiRed Probe (in DMSO)
- HRP (lyophilized)
- H₂O₂ (3%; 0.88M)
- Stop Solution
- Catalase Positive Control (lyophilized)

Target Species

Mammalian

Detection method

Absorbance (570 nm) or Fluorescence (Ex/Em 535/587 nm)

Features & Benefits

- Simple procedure; takes ~ less than 40 minutes
- Fast and convenient

Preparation

1. Sample and Positive Control Preparations:

Homogenize 0.1 gram tissues, or 10⁶ Cells, or 0.2 ml Erythrocytes on ice in 0.2 ml cold Assay Buffer; Centrifuge at 10,000 x g for 15 min 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 min 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

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assay. Keep DTT or β -ME below 5 μ M.

2. H₂O₂ Standard Curve:

Dilute 5 μ l of 0.88M H₂O₂ into 215 μ l dH₂O to generate 20 mM H₂O₂, then take 50 μ l of the 20 mM H₂O₂ and dilute into 0.95 ml dH₂O to generate 1 mM H₂O₂. Add 0, 2, 4, 6, 8, 10 μ l of 1 mM H₂O₂ solution into 96-well plate to generate 0, 2, 4, 6, 8, 10 nmol/well H₂O₂ standard. Bring the final volume to 90 μ l with Assay Buffer. Add 10 μ l Stop Solution into each well. For the fluorometric assay, dilute the standard H₂O₂ 10-fold for the standard curve (0-1 nmol range).

Note: Diluted H₂O₂ is unstable, prepare fresh dilution each time.

3. Catalase Reaction:

Add 12 μ l fresh 1 mM H₂O₂ into each well (samples, positive control, and sample HC) to start the reaction, incubate at 25°C for 30 min, and then add 10 μ l 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. Developer Mix:

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

46 μ l Assay Buffer

2 μ l OxiRed Probe

2 μ l HRP solution

Add 50 μ l of the Developer Mix to each test samples, controls, and standards. Mix well and incubate at 25°C for 10 min. 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 H₂O₂ amount to 1.5 μ l and OxiRed Probe to 0.3 μ l in the reaction; compensate the volume with Assay Buffer.

Assay Protocol

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Analysis

6. Calculation:

Signal change by catalase in sample is $\Delta A = AHC - A_{\text{sample}}$. AHC is the reading of sample High Control, A_{sample} is the reading of sample in 30 min. Plot the H₂O₂ Standard Curve. Apply the ΔA to the H₂O₂ standard curve to get B nmol of H₂O₂ decomposed by catalase in 30 min reaction. Catalase activity can be calculated:


Catalase Activity = $B / (30 * V) * \text{Sample Dilution Factor} = \text{nmol/min/ml} = \text{mU/mL}$

Where: B is the decomposed H₂O₂ amount from H₂O₂ Standard Curve (in nmol).


V is the pretreated sample volume added into the reaction well (in ml).

30 is the reaction time 30 min.

Unit definition: One unit of catalase is the amount of catalase that decomposes 1.0 μmol of H₂O₂ per min at pH 4.5 at 25 °C.

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