

Catalase Activity Colorimetric/Fluorometric Assay Kit

(Catalog #K773-100; 100 reactions; Store kit at 4 °C)

I. Introduction:

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 (H₂O₂) to water and oxygen. **BioVision'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 H₂O₂ to produce water and oxygen, the unconverted H₂O₂ 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.

II. Kit Contents:

Components	K773-100	Cap Code	Part Number
Catalase Assay Buffer	25 ml	NM	K773-100-1
OxiRed™ Probe (in DMSO)	200 µl	Red	K773-100-2
HRP (lyophilized)	1 vial	Green	K773-100-4
H ₂ O ₂ (0.88M)	25 µl	Yellow	K773-100-5
Stop Solution	1 ml	White	K773-100-6
Catalase Positive Control	2 µl	Blue	K773-100-7

III. Storage and Handling:

Store kit at 4 °C, protected from light. Warm the Assay Buffer to room temperature (RT) before use. Briefly centrifuge vials before opening. Read entire protocol before performing the assay.

IV. Reagent Reconstitution and General Consideration:

OxiRed™ Probe: Briefly warm to completely melt the DMSO solution. Store at 4 °C, protected from light. Use within two months.

HRP (lyophilized): Dissolve in 220 µl Catalase Assay Buffer. Store at 4 °C. Use within two months.

Positive Control Solution: Add 500 µl Catalase Assay Buffer to Positive Control. Divide into aliquots 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.

V. Catalase Activity Assay:

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. **Note:** 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.

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

2. H₂O₂ Standard Curve Preparation:

Dilute 5 µl of 0.88M H₂O₂ into 215 µl dH₂O to generate 20 mM H₂O₂, then take 50 µl of the 20 mM H₂O₂ and dilute into 0.95 ml dH₂O to generate 1 mM H₂O₂. Add 0, 2, 4, 6, 8, 10 µl 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 µl with Catalase Assay Buffer. Add 10 µl 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 µl 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 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. Developer Mix:

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

- 46 µl Catalase Assay Buffer
- 2 µl OxiRed™ Probe
- 2 µl HRP solution

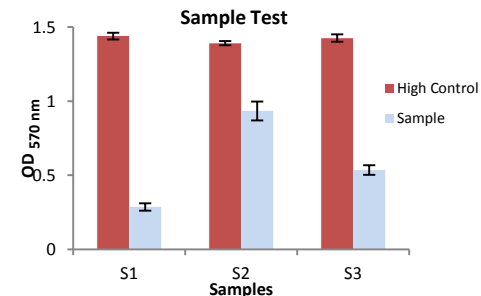
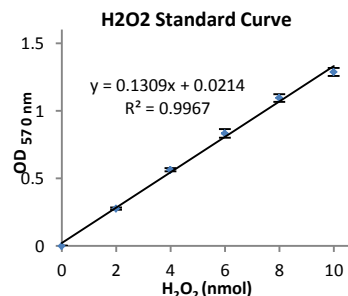
Add 50 µl of the Developer Mix to each test samples, controls, and Standards. Mix well and incubate at 25°C for 10 min. Measure OD 570 nm in a plate reader. **Note:** For low amounts of catalase, you can either increase the incubation time prior to adding the Stop Solution or use the fluorometric method. For the fluorometric method, decrease the HRP solution to 1.5 µl and OxiRed™ Probe to 0.3 µl in the reaction. Compensate the volume with Catalase Assay Buffer.

6. Calculation: Signal change by catalase in sample is $\Delta A = A_{HC} - A_{sample}$. A_{HC} is the reading of Sample High Control, A_{sample} is the reading of the 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:

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

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.



RELATED PRODUCTS:

- Colorimetric Glutathione Detection Kit
- GST Assay Kit
- Phosphatase Assay Kit
- Phosphate Assay Kit
- Pyruvate Assay Kit
- Ammonia Assay Kit
- Glucose Assay Kit
- Ethanol Assay Kit

- Glutathione Kit (GSH, GSSG and Total)
- Triglyceride Assay Kit
- ADP/ATP Ratio Assay Kit
- NAD(P)/NAD(P)H Quantification Kit
- Lactate Assay Kit/ II
- Glutamate Assay Kit
- Fatty Acid Assay Kit
- Uric Acid Assay Kit

FOR RESEARCH USE ONLY! Not to be used on humans.

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate 	<ul style="list-style-type: none"> • Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	<ul style="list-style-type: none"> • Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples 	<ul style="list-style-type: none"> • Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed • Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> • Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used 	<ul style="list-style-type: none"> • Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> • Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots 	<ul style="list-style-type: none"> • Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range 	<ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
<p>Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.</p>		