Product Information

Catalase Activity Colorimetric/Fluorometric Assay Kit

I. Kit Contents:

Components	K2177-100 100 assays	Cap Color	Part Number
Catalase Assay Buffer	25 ml	NM	K2177-C-1
OxiRed™ Probe (in DMSO)	200 µl	Red	K2177-C-2
HRP (lyophilized)	1 vial	Green	K2177-C-3
H_2O_2 (0.88M)	25 µl	Yellow	K2177-C-4
Stop Solution	1 ml	White	K2177-C-5
Catalase Positive Control	2 µl	Blue	K2177-C-6

II. Introduction:

Catalase is a ubiquitously expressed antioxidant enzyme that can be found in most of the living organisms. It catalyzes the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen. The Catalase Activity Colorimetric/Fluorometric Assay Kit offers an easy and sensitive way for measuring Catalase activity and detects high pico-unit of catalase in biological samples. In this assay, catalase first reacts with H_2O_2 to produce water and oxygen, the unconverted H_2O_2 reacts with OxiRed probe to generate a product that can be detected colorimetrically at 570 nm or fluorometrically at Ex/Em=535/587nm. Catalase activity is reversely proportional to the signal.

III. Storage and Handling:

Store kit at 4ÁC, 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.

IV. Reagent Reconstitution and General Consideration:

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

HRP: Dissolve with 220 µl Assay Buffer. Store at 4ÁC. Use within two months.

Positive Control Solution: Add 500 µl Assay Buffer to Positive Control. Aliquot and store at -20ÁC. Diluted Positive Control solution is stable for 2-3 days at 4ÁC & for 2 months at -20ÁC.

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 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 min at 4ÁC, Collect the supernatant for assay, keep on ice. Liquid samples can be tested directly. Store samples at -80ÁC 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 25ÁC 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 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 dH_2O 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 of both samples and sample HC to start the reaction, incubate at 25ÁC for 30 min, and then add 10 µl Stop Solution into each sample well 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:

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

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 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 $\Delta A = A_{HC} - A_{sample}$. A_{HC} is the reading of sample High Control, A_{Sample} is the reading of sample in 30 min. 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 min reaction. Catalase activity can be calculated:

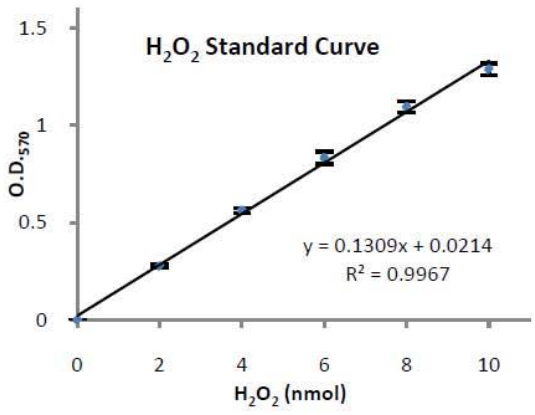
Catalase Activity = $B / (30 \times V) \times$ Sample Dilution Factor = nmol/min/ml = mU/mL

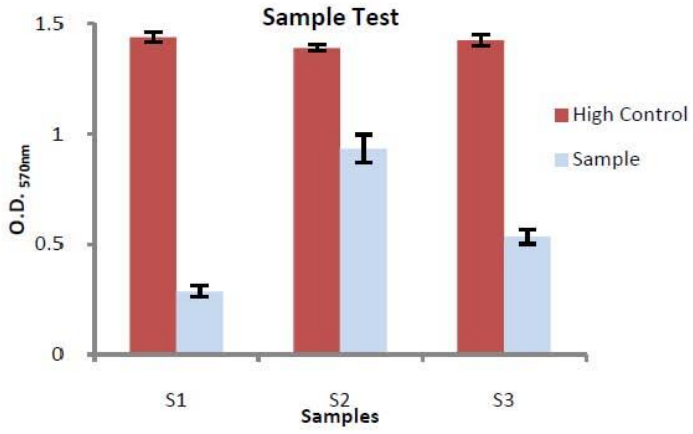
Where: B is the decomposed H_2O_2 amount from H_2O_2 Standard Curve (in nmol).

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

30 is the reaction time 30 min.

Unit definition: One unit of catalase is the amount of catalase that decomposes 1.0 µmol of H_2O_2 per min at pH 4.5 at 25°C.





General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• 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 ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• 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	• 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, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• 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	• 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 data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• 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	• 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	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• 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
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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Tel: +1-832-696-8203; Fax: +1-832-641-3177
http://www.apexbt.com/; Email: sales@apexbt.com.

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Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com