Catalase Activity Assay Kit (Catalog #K773-100; 100 reactions; Store kit at -20°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 (H202) to water and oxygen. BioVision's Catalase Assay Kit provides a highly sensitive, simple, direct and HTSready 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 detects high pU 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 (lyophilized)	2 vials	Red	K773-100-2
DMSO (anhydrous)	0.4 ml	Brown	K773-100-3
HRP (lyophilized)	1 vial	Green	K773-100-4
H_2O_2 (3%; 0.88M)	25 µl	Yelow	K773-100-5
Stop Solution	1 ml	White	K773-100-6
Catalase Positive Control (lyophilized)	1 vial	Blue	K773-100-7

III. Storage and Handling:

Store the kit at -20°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.

IV. Reagent Reconstitution and General Consideration:

OxiRed™ Probe: Dissolve each vial with 110 µl DMSO (provided, need to warm > 20°C to completely melt) prior to use; one vial is sufficient for 50 assays.
HRP Solution: Dissolve with 220 µl Assay Buffer; it is sufficient for 100 assays.
Positive Control Solution: Dissolve positive control vial into 500 µl Assay Buffer. Aliquot 100 µl per vial, store at -20°C.
Note: Warm the Assay Buffer to room temperature before use. Keep samples, HRP and Catalase on ice during the assay. All these components are stable for 2 week at 4°C or for 1 month at -20°C after reconstitution.

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. Store samples at -80°C to assay later. Add 2 - 78 µl of samples or 1 - 10 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 may interfere with the assay. Keep DTT or 2-mercaptoethanol below 5 µM.

2. H2O2 Standard Curve:

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 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:

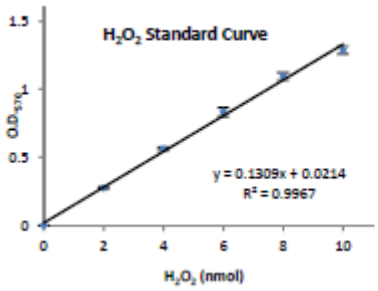
Add 12 µl fresh 1 mM H2O2 into each well of both samples and sample HC to start the reaction,
incubate at 25°C for 30 min, and then add 10 µl Stop Solution into each sample vial to stop the
reaction (Note: High Control and standard curve wells already contain Stop Solution).

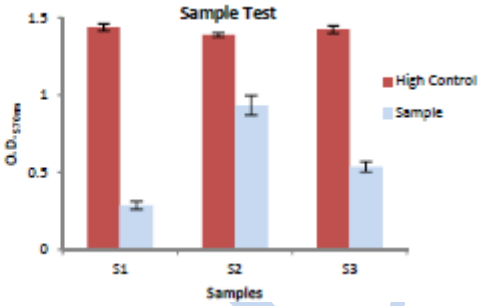
4. 4. Develop Mix: Mix enough reagents for the number of assays to be performed. For each well
prepare a 50 µl Developer Mix containing:
46 µl Assay Buffer
2 µl OxiRed™ Probe
2 µl HRP solution
Add 50 µl of the Developer Mix to each test samples, controls, and standards. Mix well and incubate at 25°C for 10 min. Measure OD 570 nm in a plate reader.
For low amounts of catalase, you can either increase the incubate time prior to adding the Stop
Solution or use the fluorometric method. For the fluorometric method, decrease the 1 mM H2O2
amount to1.5 µl and OxiRed™ Probe to 0.3 µl in the reaction; compensate the volume with Assay Buffer.

5. Calculation: Signal changes by catalase in sample is ΔA = AHC – ASample. AHC is the reading of
sample High Control, ASample 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:

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

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 decomposes 1.0 μmol of H2O2 per min at pH 4.5 at 25 °C.





VI. Related Products:

Colorimetric Glutathione Detection Kit Glutathione Kit (GSH, GSSG and Total)
GST Assay Kit Triglyceride Assay Kit
Phosphatase Assay Kit ADP/ATP Ratio Assay Kit
Phosphate Assay Kit NAD(P)/NAD(P)H Quantification Kit
Pyruvate Assay Kit Lactate Assay Kit/ II
Ammonia Assay Kit Glutamate Assay Kit
Glucose Assay Kit Fatty Acid Assay Kit
Ethanol Assay Kit Uric Acid Assay Kit
Glycogen Assay Kit Phosphate Assay Kits

GENERAL TROUBLESHOOTING GUIDE

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• 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.		