

# Catalase 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 (H<sub>2</sub>O<sub>2</sub>) 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 H<sub>2</sub>O<sub>2</sub> to produce water and oxygen, the unconverted H<sub>2</sub>O<sub>2</sub> 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.

## II. Kit Contents:

Components	K773-100	Cap Code	Part No.
Catalase Assay Buffer	25 ml	NM	K773-100-1
OxiRed™ Probe	2 vials	Red	K773-100-2
DMSO (anhydrous)	0.4 ml	Brown	K773-100-3
HRP	1 vial	Green	K773-100-4
H <sub>2</sub> O <sub>2</sub> (3%; 0.88M)	25 µl	Yellow	K773-100-5
Stop Solution	1 ml	White	K773-100-6
Catalase Positive Control	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, needs hand-warm up to 18°C to become liquid) 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.

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 10<sup>6</sup> Cells, or 0.2 ml Erythrocytes on ice in 0.4-1.0 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 for storage.

Add 2-78 µl 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; bring total volume to 78 µl with Assay Buffer. Add 10 µl of Stop Solution into the sample High Control, 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. H<sub>2</sub>O<sub>2</sub> Standard Curve:

Dilute 5 µl of 0.88M H<sub>2</sub>O<sub>2</sub> into 215 µl DiH<sub>2</sub>O to generate 20 mM H<sub>2</sub>O<sub>2</sub>, then take 50 µl of the 20 mM H<sub>2</sub>O<sub>2</sub> and dilute into 0.95 ml DiH<sub>2</sub>O to generate 1 mM H<sub>2</sub>O<sub>2</sub>. Add 0, 2, 4, 6, 8, 10 µl of 1 mM H<sub>2</sub>O<sub>2</sub> solution into 96-well plate to generate 0, 2, 4, 6, 8, 10 nmol/well H<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> 10 more folds for the standard curve. Diluted H<sub>2</sub>O<sub>2</sub> is unstable, prepare fresh dilution each time.

### 3. Catalase Reaction:

Add 12 µl fresh 1 mM H<sub>2</sub>O<sub>2</sub> into each well of both samples and sample High Controls 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. Develop Mix:

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

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

Add 50 µl of the Develop Mix to each test samples, controls, and standards. Mix well and incubate at 25°C for 10 min. Measure O.D. 570 nm in a plate reader.

For low amount catalase, you can either increase the incubate time prior to adding the Stop Solution or use fluorometric method. For the fluorimetric method, decrease the 1 mM H<sub>2</sub>O<sub>2</sub> amount to 1.5 µl and OxiRed™ Probe to 0.3 µl in the reaction.

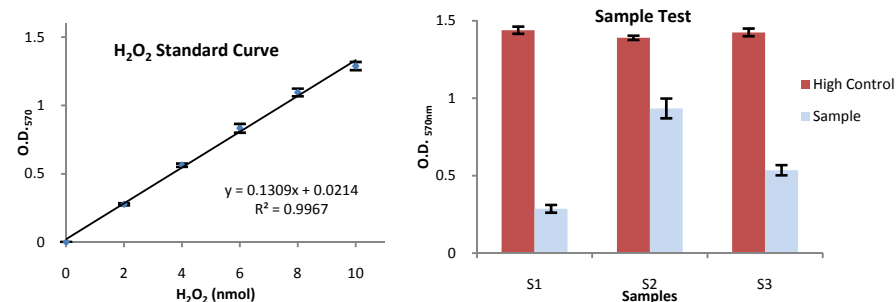
### 5. Calculation:

Signal changes 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<sub>2</sub>O<sub>2</sub> Standard Curve. Apply the  $\Delta A$  to the H<sub>2</sub>O<sub>2</sub> standard curve to get B nmol of H<sub>2</sub>O<sub>2</sub> decomposed by catalase in 30 min reaction. Catalase activity can be calculated:

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

**Where:** B is the decomposed H<sub>2</sub>O<sub>2</sub> amount from H<sub>2</sub>O<sub>2</sub> Standard Curve (in nmol).  
V is the pretreated sample volume added into the reaction well (in ml).  
30 is reaction time 30 min.

**Unit definition:** One unit of catalase is the amount of catalase decomposes 1.0 µmol of H<sub>2</sub>O<sub>2</sub> per min at pH 4.5 at 25 °C.



## VI. 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  
Glycogen 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  
Phosphate Assay Kits