Catalase Activity Assay (Colorimetric/ Fluorometric) Catalog: TBS2006
(100 Assays, Store at -20°C)

Two-steps Sensitive Quantitation for Catalase activity in Serum, Plasma, Urine and Other bio-samples

DESCRIPTION

Catalase (EC 1.11.1.6) is an ubiquitous antioxidant which catalyzes the conversion of hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2). H_2O_2 is an oxidative stress inducer that can cause damage cells at relatively low concentrations. H_2O_2 is also precursor to certain oxidant radical species (e.g. hydroxyl radical). Catalase plays a crucial role to prevent oxidative damage to the cells.

Tribioscience catalase Assay Kit provides a rapid, simple, reproducible, and sensitive tool for catalase activity determination in plasma, serum, urine, and other samples. The catalase activity assay uses enzyme reactions to produce color and fluorescence with the probe. The color intensity of the reaction at 570 nm or fluorescence intensity at λex/em = 530/590 nm is directly proportional to catalase concentration in the sample. The fluorometric assay is more sensitive than colorimetric assay.

APPLICATIONS

Direct Assays: Catalase enzyme activity in serum, plasma, urine, and other bio-samples.

KEY FEATURES

Flexible: Suitable for colorimetric and fluorometric methods.
Accurate: Use 25 µL samples, detection ranges are 1.95-31.25 U/mL in 96-well plate the assay.
Simple and high-throughput: Two-steps procedure: just load-incubate-adding-incubate-read. Kits can be used as a robust method.

KIT CONTENTS

Component	Part Size
Assay Buffer	10 mL
Enzyme substrate (10x)	0.3 mL
Red Probe	0.12mL
H_2O_2 Standard (400 µM)	0.3 mL
Enzyme positive control	60 µL
Enzyme mix	0.12 mL

STORAGE AND HANDLING

Store kit at -20°C. Shelf life of 1year. Protect from light. Shipment with blue ice. Avoid frozen and thaw circles.

FLOROMETRIC PROTOCOL

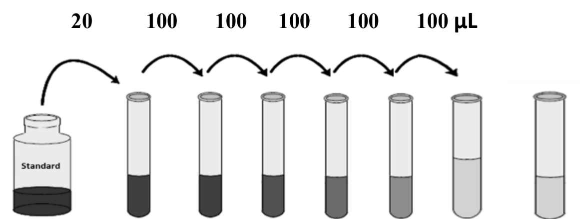
Ensure the Reagent is at room temperature before use. Keep samples and enzyme on ice before the assay. It is recommended that all standards and samples be duplicated in the assay.

Sample Preparations:
Serum, Plasma, other body fluid, or cell culture supernatant can be measured directly by a series of dilutions of the sample (1/2, ¼, or 1/8). Solid samples, such as tissues, can be first homogenized and extracted with PBS for 1 h at 4°C, followed by centrifugation at 10,000 g for 10 min. The clear supernatants then can be measured as described for liquid samples. Add 25µL test samples directly into 96-well black plate.

Assay Procedures

1. Add 25 µL of sample or positive control to each well of a black microplate in duplicate manner *(Note: the black microplate is for fluorescence detection)*.
2. Diluting 1x substrate: for example, by adding 22.5 µL assay buffer with 2.5 µL of 10x substrate for one well, then add 25 µL of enzyme substrate (1x) to each well containing the test samples and positive control. Tap plate lightly to mix.
3. Incubate at 37°C for 30 minutes gently shaking.
4. Standard Curve Preparations as shown as Fig. 1:
   Label 1.5mL tube from Std1 to 8. As below the diagram. 40 µM H_2O_2 Standard by take 20 µl of 400 µM H_2O_2 + 180 µL assay buffer solution as Std1 (40 µM).
5. Add 100uL of 1x Assay Buffer to Std2 to 8.
6. Make 2x series dilution from Std2 through 7 by transferring 100µL higher concentration of the standard solution to the next one. Std8 is 1x Assay Buffer alone as a standard 0 and **Blank**. The standard concentration range is 40, 20, 10, 5, 2.5, 1.25, 0.625 & 0 µM or 1000, 500, 250, 125, 62.5, 31.25, 15.625, 0 pmol/well

Fig.1: Diagram of Standard Preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	180	100	100	100	100	100	100	100
Addition	400 µM	Std1	Std2	Std3	Std4	Std5	Std6	
Addition (µL)	20	100	100	100	100	100	100	
Final Conc (µM)	40	20	10	5	2.5	1.25	0.625	0

7. Add 25 µL standards solution to appropriate wells in duplicate manner.
8. **Premix** working solution: 48 µL Assay Buffer with 1 µL enzyme mix, and 1µL red probe for 50 µL of each well.
9. Add 50 µL of **Premix** working solution to each well containing the Standard and test samples and positive control. Tap plate lightly to mix.
10. Incubate at 37°C for 30 minutes, protect from light.
11. Measure the fluorescence intensity using a microplate reader, equipped for excitation of 530nm and emission detection at 590 nm. *(Note: Because the assay is continuous (not terminated), fluorescence or absorbance may be measured at multiple time points to follow the kinetics of the reactions).*

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COLORIMETRIC PROCEDURE

1. The colorimetric assay is similar with the fluorometric assay. Transfer 25 µL of positive control and samples into separate wells of a 96-well clear white plate.
2. Add 25 µL enzyme substrate (1x, e.g. 24µL of assay buffer + 1µL of substrate 5x for one well) to each well containing the test samples and positive control.
3. Tap plate lightly to mix.
4. Incubate at 37°C for 30 minutes gently shaking.
5. Prepare the standard using fluorometric assay **Fig.1** as reference, e.g. 200 µM H_2O_2=100 µL of 400 µM H_2O_2+ 100 µL of assay buffer as Std1, then add 100uL of 1x Assay Buffer to Std2 to 8. Make 2x series dilution from Std2 through 7 by transferring 100µL higher concentration of the standard solution to the next one. Std8 is 1x Assay Buffer alone as a standard 0 and **Blank**. The standard concentration range is 200, 100, 50, 25, 12.5, 6.25, 3.125, 0 µM or 5000, 2500,1250, 625, 312.5, 156.25, 78.125 & 0 pmol/well.
6. Add 25 µL of standards solution to appropriate wells in duplicate manner.
7. **Premix** working solution: 48 µL Assay Buffer with 1 µL enzyme mix, and 1µL red probe for 50 µL of each well.
8. Ad 50 µL of **Premix** working solution to each well containing the Standard and test samples and positive control (see fluorometric procedure), tap plate to mix. Incubate 30-60 min at 37°C.
9. Read OD value at 570 nm (550-585 nm).

Calculation

The standard values subtract the blank value (0 µM Standard) and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the catalase concentration of the sample using the equation obtained from the linear regression of the standard curve.

Catalase Activity (U/L) = DF * (ODBLANK – OD SAMPLE)/ (t * Slope)

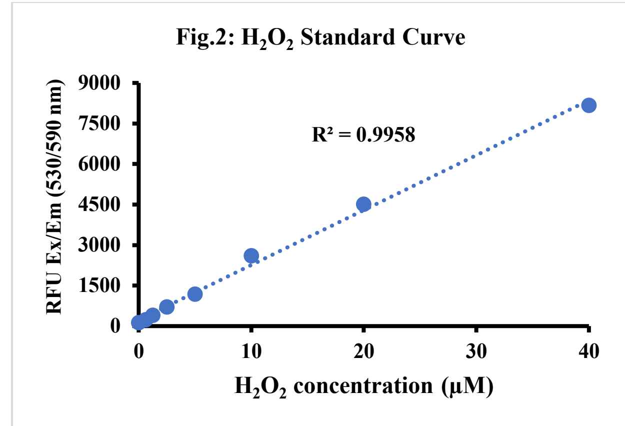
Where: R sample and R blank are optical density or fluorescence intensity readings of the sample and blank, respectively. DF is the sample dilution factor. Slope is the linear regression fit of the standard points and t is the reaction time (30 or 60 min).

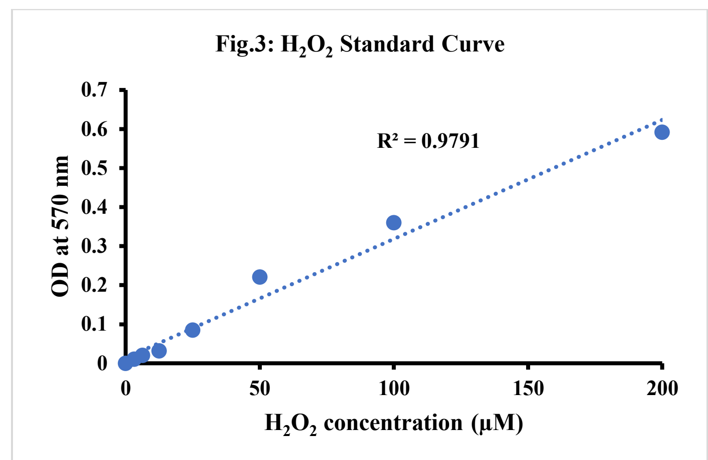
Note1: If unknown sample results are over standard curve range, dilute samples with assay buffer, and repeat the assay.

Unit definition: 1 Unit (U) will catalyze the conversion of 1 µmole of substrate to H_2O per min at 37°C.

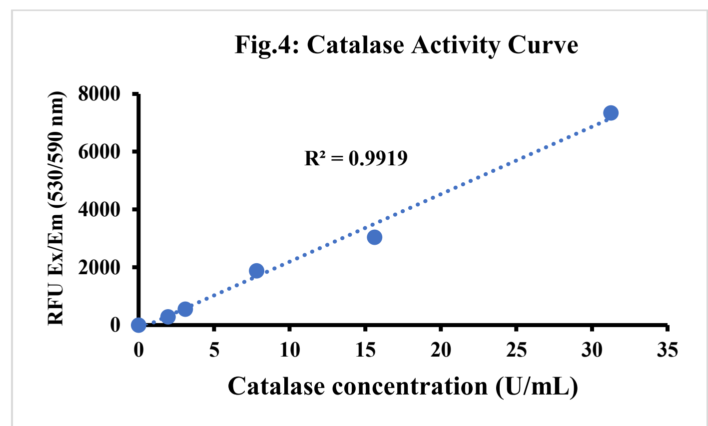
TYPICAL DATA

The typical data is provided for only demonstration reference of standard curve shown in Fig.2 for fluorometric assay and Fig.3 for colorimetric assay.





The catalase activity assay as shown in **Fig.4**



RELATED PRODUCTS

Resazurin Cell Viability Kit (TBS2001)
ATP Colorimetric/Fluorometric Assay (TBS2010)
ADP Colorimetric/Fluorometric Assay Kit (TBS2020)
CCK-8 Cell Viability Assay (TBS2022)
Thiol Fluorometric Assay (TBS2026)
Caspase-3 Colorimetric Assay kit (TBS2030)

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