MANUAL

RayBio® Catalase Activity Assay Kit (Colorimetric)

Catalog #: MA-CAT

ISO 13485:2016

Introduction

Catalase is a ubiquitous antioxidant enzyme found in both mammalian and non-mammalian aerobic cells with a cytochrome system. Its primary function is to facilitate the decomposition of hydrogen peroxide (H_2O_2) into water and oxygen. Hydrogen peroxide can be highly detrimental to cells, as its accumulation can cause oxidative damage to crucial cellular components like DNA, proteins, and lipids, ultimately leading to mutagenesis and cell death. Catalase plays a vital role in safeguarding cells against this oxidative damage, and its involvement in oxidative stress-related diseases has been extensively studied.

The RayBio® Catalase Activity Assay Kit provides a user-friendly, reproducible, rapid, and highly sensitive method for measuring catalase activity in various biological liquid samples, including plasma, serum, and cell lysates. This assay comprises two sequential reactions. Initially, catalase efficiently breaks down hydrogen peroxide (H_2O_2) into water and oxygen. Following this, sodium azide is introduced to halt catalase activity. In the subsequent reaction, any residual H_2O_2 in the mixture participates in a coupling reaction involving a Colorimetric Probe and a Horseradish Peroxidase (HRP) catalyst. The resulting coupling product is then measured at 520nm, allowing for a precise quantification of the remaining hydrogen peroxide in the reaction mixture. This assay effectively evaluates catalase activity.

Storage

The entire kit may be stored at < -20 °C for up to 6 months from the date of shipment. For prepared reagent storage, see table below.

Component	Size / Description	Storage After Preparation
Microplate (Item A)	A 96-well (12 strips x 8 wells) plate	RT*
Sample Buffer	10 ml	2–8 °C, 4 weeks
Assay Buffer	25 ml	2–8 °C, 4 weeks
Hydrogen Peroxide	1 amber vial (100 µl of 8.82M)	Do not store and resuse
Catalase Quencher	10 ml (amber bottle)	2–8 °C, protect from light, 4 weeks
Catalase Standard	2 vials (10 µl of 124,000 U/mL)	Do not store and resuse
Horseradish Peroxidase Concentrate	2 amber vials (Lyophilized)	Do not store and resuse
Colorimetric Probe	2 amber vials (Lyophilized)	Do not store and resuse

RT = room temperature

**Return unused wells to the pouch containing desiccant pack, reseal along entire edge.*

Additional Materials Required

1. Microplate reader capable of measuring absorbance at 520 nm
2. Precision pipettes to deliver 2 µl to 1 ml volumes
3. Multi-channel pipettes to deliver 20 µl to 200 µl volumes
4. Tubes to prepare sample dilutions
5. 50 ml conical tubes

Sample Tips and General Considerations

NOTE: Optimal methods of sample preparation will need to be determined by each researcher empirically based on researched literature and knowledge of the samples.

- If not using fresh samples, freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot samples prior to initial storage.
- It is strongly recommended to add a protease inhibitor cocktail to cell and tissue lysate samples.
- Avoid sonication of 1 ml or less as this can quickly heat and denature proteins.
- Most samples will not need to be concentrated. If concentration is required, a spin column concentrator with a chilled centrifuge is recommended.

1. Cell lysates can be prepared as follows:

For attached cells, remove supernatant from cell culture, wash cells twice with cold 1X PBS (for suspension cells, pellet the cells by spinning down the cells at 1,000 x g for 10 min) making sure to remove any remaining PBS before adding lysis buffer. Solubilize the cells at $2x10^7$ cells/ml in lysis buffer containing protease inhibitors. Pipette up and down to resuspend cells and rock the lysates gently at 2–8 °C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 14,000 x g for 10 minutes.
It is recommended that sample protein concentrations should be determined using a total protein assay. Lysates should be used immediately or aliquot and stored at -70 °C. Thawed lysates should be kept on ice prior to use.
General tips for preparing lysate samples can be viewed on the online Resources page of the website: https://www.raybiotech.com/tips-on-sample-preparation/

2. Plasma samples:

Collect blood with an anticoagulant such as citrate, EDTA or oxalate and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer. Sample should be tested immediately or frozen at -80°C for storage.

3. Serum samples:

Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C for storage.

Note: If the calculated Catalase concentration of the sample is higher than 100 U/mL, dilute the sample in sample buffer and repeat the assay.

Reagent Preparation

REAGENT	PREPARATION
Microplate (Item A)	No preparation
Sample Buffer	
Assay Buffer	
Catalase Quencher	
Hydrogen Peroxide	Add 4.54 µL Hydrogen Peroxide (H_2O_2) into 1995.46 µL Assay Buffer to get 20mM H_2O_2. Mix well. Then add 250 µL of 20mM H_2O_2 into 4750 µL Assay Buffer to get 1mM H_2O_2. This is the **Hydrogen Peroxide Working Solution**. Don't store this working solution.
Catalase Standard	See "Standard Preparation" section.
Horseradish Peroxidase Concentrate	For each vial, reconstitute with 1 ml Assay Buffer.
Colorimetric Probe	For each vial, reconstitute with 1 ml Assay Buffer.
Chromogenic Working Solution	Add 1 mL reconstituted Horseradish Peroxidase Concentrate and 1 mL reconstituted Colorimetric Probe into 6 mL Assay Buffer to make **Chromogenic Working Solution**. This is enough to assay 55 wells. Prepare the additional vial as needed. The solution is stable for 1 day at 2-8°C in the dark.

Standard Preparation

To prepare a dilution series of Catalase Standard in the concentration range of 0 U/mL – 100 U/mL (see Table below), *Note:* Catalase is unstable and should be kept on ice while being used. Use diluted standards within 60 minutes of preparation. Do not store diluted Catalase Standard solutions.

1. Label 8 microtubes #1 through 8 which with the following concentrations: 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0 U/mL.
2. Pipette 495 µL of Sample Buffer into labeled tube #1, and 100 µL Sample Buffer into labeled tubes #2 – tube #8.
3. Pipette 3.22 µL Catalase Standard into 36.78 µL Sample Buffer to make the 10,000 U/mL Catalase Standard stock.
4. Pipette 5 µL 10,000 U/mL Catalase Standard stock into tube #1 to make 100 U/mL Catalase Standard.
5. To make the 50 U/mL standard, pipette 100 µL of tube #1 into the tube labeled #2. Mix thoroughly.
6. Repeat this step with each successive concentration, preparing a dilution series as shown in the Table below. Each time, use 100 µl of the prior concentration until the 1.5625 U/mL is reached. Mix each tube thoroughly before the next transfer. Standards should be prepared freshly, mixed thoroughly, and used immediately.

Labeled Tubes	Catalase Standard (µL)	Sample Buffer (µL)	Standard Activity (U/mL)
1	5 µL of 10,000 U/mL	495 µL	100
2	100 µL of Tube #1	100 µL	50
3	100 µL of Tube #2	100 µL	25
4	100 µL of Tube #3	100 µL	12.5
5	100 µL of Tube #4	100 µL	6.25
6	100 µL of Tube #5	100 µL	3.125
7	100 µL of Tube #6	100 µL	1.5625
8	0 µL	100 µL	0

Assay Procedure

Each catalase standard and samples should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

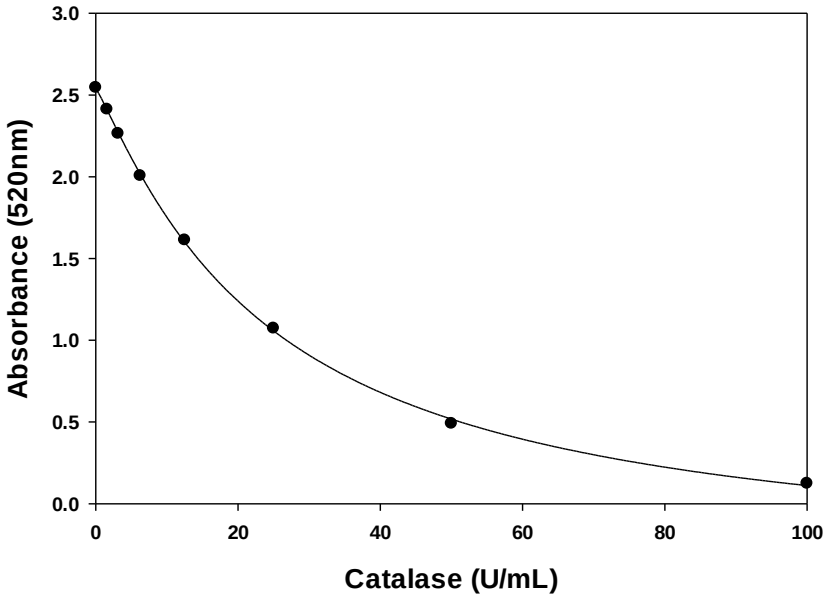
1. Add 20 µL of the diluted Catalase Standards or unknown samples to a 96-well microtiter plate.
2. Add 30 µL of the Hydrogen Peroxide Working Solution (1mM) to each well (Use multi-channel pipettes if possible). Mix thoroughly and incubate at room temperature for exactly 1 minute.
3. Stop the reaction by adding 50 µL of the Catalase Quencher into each well and mix thoroughly for 5 minutes.
4. Add 150 µL of the Chromogenic Working Solution to each well. Incubate the plate at room temperature for 15 minutes gently mixing.
   Note: Perform this step within 15 minutes after stopping the enzymatic reaction (Step 3).
5. Measure the absorbance at 520nm using a plate reader.

Calculation of Results

1. Calculate the average absorbance values for each set of standards and samples.
2. Plot the standard curve using SigmaPlot software (or other software which can perform four-parameter logistic regression models), with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit curve through the standard points.
3. Use the mean absorbance value for each sample to determine the corresponding concentration from the standard curve.

Note: If the calculated Catalase concentration of the sample is higher than 100 U/mL, dilute the sample in sample buffer and repeat the assay.

A. Typical Data



These standard curves are for demonstration only. A standard curve must be run with each assay.

B. Reproducibility

Intra-assay Precision (Precision within an assay):
To assess intra-assay precision, 16 wells per sample (total of 4 samples) were tested on a single plate. The intra-assay coefficient of variation was found to be 5.1%.

Inter-assay Precision (Precision between assays):
To assess inter-assay precision, 4 samples were tested in separate assays (n=4). The inter-assay coefficient of variation was found to be 10.4%.

This product is for research use only.