Amplex Red Catalase Activity Assay Kit

Introduction

Catalase is an enzyme that is ubiquitous in cells, mainly found in the liver, kidneys, and red blood cells. Its main function is to catalyze the decomposition of hydrogen peroxide (H_2O_2) to produce water and oxygen, thereby effectively reducing the concentration of H_2O_2 in cells. H_2O_2 is a strong oxidant that can trigger oxidative stress, damage cells and tissues, and lead to cell dysfunction and the occurrence of a variety of diseases. Therefore, catalase plays an important role in maintaining the stability of the intracellular environment and protecting cells from oxidative damage. Studying the activity of catalase and its regulatory mechanism is of great significance for understanding the antioxidant defense mechanism of cells and exploring the occurrence and development of diseases related to oxidative stress.

Amplex Red Catalase Activity Assay Kit provides a sensitive and simple method for measuring catalase activity. The working principle of this kit is based on catalase-catalyzed decomposition of H_2O_2. Catalase catalyzes H_2O_2 into water and oxygen (O_2). Under the presence of horseradish catalase (HRP), the remaining unreacted H_2O_2 can react with the Amplex Red reagent to form highly fluorescent resorufin. Therefore, as catalase activity increases, the fluorescence signal of resorufin decreases. Catalase activity in the sample can be indirectly detected by setting a no-catalase control.

Components and Storage

Components \ Size	400 Tests	Storage
Amplex Red Reagent	2 vials	-20°C away from light
Dimethylsulfoxide (DMSO)	500 µL	-20°C
Horseradish peroxidase	1 vial	-20°C away from light
Hydrogen peroxide	500 µL	-20°C
5X Reaction Buffer	20 mL	-20°C
Catalase	1 vial	-20°C
Shipping: Blue ice	**Shelf life: 1 year**	

Protocol

1. Preparation before the experiment:

   1) Amplex Red (10 mM) Preparation: Dissolve 1 vial of Amplex Red in 100 µL of DMSO to make the Amplex

Red (10 mM). 1 vial of Amplex Red (10 mM) is sufficient for 200 tests. Prepared Amplex Red (10 mM) needs to be stored at -20°C protected from light.

2) 1× Reaction Buffer Preparation: Dilute an appropriate amount of 5× Reaction Buffer to 1 × with ddH_2O. For example, dilute 5 mL of 5× Reaction Buffer in 20 mL of ddH_2O.

3) Horseradish Peroxidase (100 U/mL) Preparation: Dissolve 1 vial of Horseradish Peroxidase in 200 µL of 1× Reaction Buffer to make the Horseradish Peroxidase (100 U/mL). Unused Horseradish Peroxidase (100 U/mL) should be aliquoted and stored at -20°C in the dark.

4) Hydrogen Peroxide (20 mM) Preparation: Dilute an appropriate amount of Hydrogen Peroxide with 1× Reaction Buffer to prepare Hydrogen Peroxide (20 mM). For example, dilute 23 µL of Hydrogen Peroxide in 977 µL of 1× Reaction Buffer and mix gently. Hydrogen Peroxide (20 mM) is unstable and should to be prepared freshly every time.

5) Catalase (1000 U/mL) preparation: dissolve a vial of Catalase with 100 µL ddH_2O to make the Catalase (1000 U/mL). Prepared Catalase (1000 U/mL) needs to be aliquoted and stored at -20°C.

2. Sample Preparation:

1) Serum or plasma can be used directly.

2) For cells or tissues, homogenization in an appropriate manner and centrifugation to obtain the supernatant. Then place the supernatant on ice for later use.

***Note:** Try to use fresh samples for experiments.

3. Standard curve preparation: Obtain Catalase (100 U/mL) by 10-fold dilution of Catalase (1000 U/mL) with 1× Reaction Buffer. Refer to the following table to obtain different Catalase standards. 25 µL of standard is required for each reaction, and it is recommended to prepare at least two parallel wells for each set of standards.

Standard #	Catalase Solution	1× Reaction Buffer	Final Catalase concentration
1	0 µL	25 µL	0 mU/mL
2	6.25 µL of 1 U/mL	18.75 µL	62.5 mU/mL
3	12.5 µL of 1 U/mL	12.5 µL	125 mU/mL
4	2.5 µL of 10 U/mL	22.5 µL	250 mU/mL
5	5 µL of 10 U/mL	20 µL	500 mU/mL
6	10 µL of 10 U/mL	15 µL	1000 mU/mL
7	2 µL of 100 U/mL	23 µL	2000 mU/mL
8	4 µL of 100 U/mL	21 µL	4000 mU/mL
9	8 µL of 100 U/mL	17 µL	8000 mU/mL

*Note: The final concentration Catalase in the table refers to the concentration in the final assay (100 μL).

4. Catalase activity assay:

1) Take 1-25 μL of the sample and adjust the volume to 25 μL with 1× Reaction Buffer. 25 μL is required for each reaction, and it is recommended to prepare at least two parallel wells per sample.

2) Transfer different standards and samples to 96-well plates. Each well needs 25 μL.

3) Prepare Hydrogen Peroxide (40 μM) by mixing 10 μL of Hydrogen Peroxide (20 mM) and 4.99 mL of 1× Reaction Buffer.

4) Add 25 μL of Hydrogen Peroxide (40 μM) to each well, mixed and incubated for 30 minutes at room temperature.

5) Preparation of the Reaction mix: Refer to the following table to prepare the Reaction mix, the final concentrations of Amplex Red and Horseradish Peroxidase are 100 μM and 0.4 U/mL, respectively. 50 μL of Reaction mix is required per well.

	Reaction mix
Amplex Red (10 mM)	50 μL
Horseradish Peroxidase (100 U/mL)	20 μL
1× Reaction Buffer	4.93 mL
Total volume	5 mL

6) Add 50 μL of Reaction mix to each well and mix well.

7) Detect the kinetic curve (Ex/Em=570/595 nm) for 30 min.

*Note: It is recommended to use kinetic mode for detection, or you can use the endpoint method after adding the Reaction mix and incubating for 30 minutes in the dark.

5. Data analysis: Subtract the samples and standards value from that of the no-Catalase control (standard #1) to obtain the correct values for Catalase-catalyzed reactions. Then establish the correct standard curve, and apply the correct samples value to the standard curve to get the Catalase activity in the sample.

Note

1. Before the formal experiment, it is recommended to perform several dilutions of the sample to ensure the readings are within the standard value range.

2. For your safety and health, please wear lab coats and gloves during the experiment.

3. For research use only. Not to be used in clinical diagnostic or clinical trials.

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