Amplite™ Fluorimetric Catalase Assay Kit *Red Fluorescence*

Catalog number: 11306
Unit size: 200 Tests

Component	Storage	Amount
Component A: Amplite™ Red	Freeze (<-15 °C), Dessicated, Avoid Light	1 vial
Component B: H2O2	Freeze (<-15 °C), Avoid Light	1 vial (3% stabilized solution, 200 µL)
Component C: Assay Buffer	Refrigerate (2-4 °C)	1 bottle (20 mL)
Component D: Horseradish Peroxidase	Freeze (<-15 °C), Dessicated, Avoid Light	1 vial (20 units)
Component E: Catalase Standard	Freeze (<-15 °C), Avoid Light	1 vial (1000 U/mL, 50 µL)
Component F: DMSO	Freeze (<-15 °C), Avoid Light	1 vial (200 µL)

OVERVIEW

Catalase is a common antioxidant heme-containing redox enzyme found in nearly all living organisms that are exposed to oxygen. The enzyme is concentrated in the peroxisome subcellular organelles. Hydrogen peroxide is an ROS that is a toxic product of normal aerobic metabolism and pathogenic ROS production involving oxidase and superoxide dismutase reactions. By preventing the excessive buildup of H2O2, catalase allows important cellular processes which produce H2O2 as a by-product to take place safely. The Amplite™ Fluorimetric Catalase Assay Kit provides a quick and sensitive method for the measurement of catalase activity. It can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. Catalase reacts with H2O2 to produce water and oxygen (O2). Amplite™ Red also reacts with H2O2 to generate a red fluorescent product. Therefore the reduction in fluorescence intensity is proportional to catalase activity. The Amplite™ Red substrate used in the assay enables a dual recordable mode. The fluorescent signal can be easily read by either a fluorescence microplate reader at Ex/Em = 540/590 nm or an absorbance microplate reader at ~576 nm. With the Amplite™ Fluorimetric Catalase Assay Kit, we have detected as little as 30 mU/mL catalase in a 100 µL reaction volume.

AT A GLANCE

Protocol summary

1. Prepare Catalase standards and/or test samples (50 µL)
2. Add H_2O_2 Assay Buffer (50 µL)
3. Incubate at room temperature for 10 - 30 minutes
4. Add Catalase Assay Mixture (50 µL)
5. Incubate at room temperature for 10 - 30 minutes
6. Monitor fluorescence increase at Ex/Em = 540/590 nm (Cutoff = 570nm)

Important Thaw all the kit components at room temperature before starting the experiment. The component A is unstable in the presence of thiols such as DTT and β-mercaptoethanol. The final concentration of the thiols higher than 10 µM would significantly decrease the assay dynamic range. NADH and glutathione (reduced form: GSH) may interfere with the assay.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Amplite™ Red Substrate stock solution (200X):
Add 65 µL of DMSO (Component F) into the vial of Amplite™ Red (Component A) to make 200X Amplite™ Substrate stock solution. The stock solution should be used promptly.

2. HRP stock solution (100 U/mL):
Add 200 µL of Assay Buffer (Component C) into the vial of Horseradish Peroxidase (Component D) to make 100 U/mL HRP stock solution.

3. H_2O_2 stock solution (10 mM):
Add 10 µL of 3% H_2O_2 (0.88 M, Component B) into 870 µL of Assay Buffer (Component C) to make 10 mM H_2O_2 stock solution.

Note The diluted H_2O_2 stock solution is not stable. The unused portion should be discarded.

4. H_2O_2 assay buffer (1X):
Add 5 µL of 10 mM H_2O_2 stock solution into 5 mL of Assay Buffer (Component C) to make 1X H_2O_2 assay buffer.

5. Catalase standard solution (2 U/mL):
Add 2 µL of 1000 U/mL Catalase Standard (Component E) into 1000 µL of Assay Buffer (Component C) to make 2 U/mL Catalase standard solution.

PREPARATION OF STANDARD SOLUTION

Catalase standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11306

Take 2 U/mL Catalase standard solution (CS7) and perform 1:2 serial dilutions to get serially diluted Catalase standard (CS6 - CS1) with Assay Buffer (Component C).

PREPARATION OF WORKING SOLUTION

Add 25 µL of 200X Amplite™ Red substrate stock solution and 15 µL of 100 U/mL HRP stock solution into 5.0 mL of Assay Buffer (Component C) and mix well to prepare Amplite™ Red working solution.

Note Keep from light.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Catalase standards and test samples in a solid black 96-well microplate. CS= Catalase Standards (CS1 - CS7, 0.031 to 2 U/mL), BL=Blank Control, TS=Test Samples.

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com

BL	BL	TS	TS
CS1	CS1	...	...
CS2	CS2	...	...
CS3	CS3		
CS4	CS4		
CS5	CS5		
CS6	CS6		
CS7	CS7		

Table 2. Reagent composition for each well.

Well	Volume	Reagents
CS1 - CS7	50 µL	Serial Dilution (0.031 to 2 U/mL)
BL	50 µL	Assay Buffer (Component C)
TS	50 µL	test sample

1. Prepare Catalase standards (CS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.
2. Add 50 µL of H_2O_2 assay buffer to each well of Catalase standard, blank control, and test samples to make the total Catalase assay volume of 100 µL/well. For a 384-well plate, add 25 µL of H_2O_2 assay buffer into each well instead, for a total volume of 50 µL/well.
3. Incubate the reaction at room temperature for 15 to 30 minutes, protected from light.
4. Add 50 µL of Amplite™ Red working solution into each well of Catalase standard, blank control, and test samples to make the total assay volume of 150 µL/well. For a 384-well plate, add 25 µL of Amplite™ Red working solution into each well instead, for a total volume of 75 µL/well.
5. Incubate the reaction at room temperature for 15 to 30 minutes, protected from light.
6. Monitor the fluorescence increase with a fluorescence plate reader at Excitation = 540 ± 10, Emission = 590 ± 10 nm (Cutoff = 570 nm) (optimal Ex/Em = 540/590 nm).

Note The contents of the plate can also be transferred into a white clear bottom plate and read by an absorbance microplate reader at the wavelength of 576 ± 5 nm. The absorption detection has lower sensitivity compared to fluorescence reading.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Catalase samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

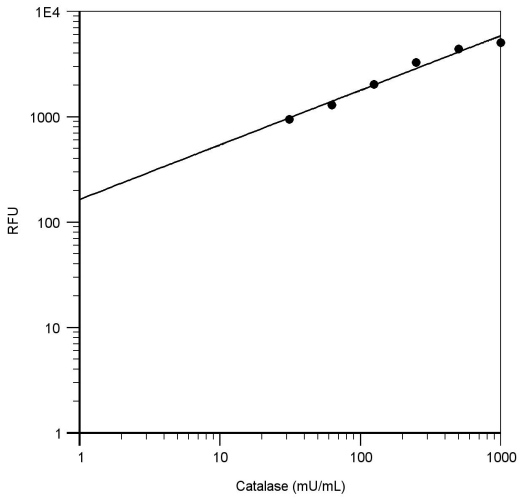

Figure 1.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.