Amplite™ Fluorimetric Catalase Assay Kit

Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 11306 (200 assays)	Keep in freezer. Avoid exposure to light.	Fluorescence microplate readers

Introduction

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 H_2O_2, catalase allows important cellular processes which produce H_2O_2 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 H_2O_2 to produce water and oxygen (O_2). Amplite™ Red also reacts with H_2O_2 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.

Kit Key Features

Sensitive:	Detect as low as 30 mU/mL catalase.
Continuous:	Easily adapted to automation without a separation step.
Convenient:	Formulated to have minimal hands-on time.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Components	Amount
Component A: Amplite™ Red	1 vial
Component B: H_2O_2	1 vial (3% stabilized solution, 200 µL)
Component C: Assay Buffer	1 bottle (20 mL)
Component D: Horseradish Peroxidase	1 vial (20 units)
Component E: Catalase Standard	1 vial (1000 U/mL, 50uL)
Component F: DMSO	1 vial (200 µL)

Assay Protocol for One 96-well Plate

Brief Summary

Prepare catalase standards and/or test samples (50 µL) → Add H_2O_2 Assay Buffer (50 µL) → Incubate at room temperature for 10-30 minutes → Add Catalase Assay Mixture (50 µL) → Incubate at room temperature for 10-30 minutes → Monitor fluorescence increase at Ex/Em = 540/590 nm

Note: Thaw all the kit components at room temperature before starting the experiment.

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1. Prepare stock solutions:

1.1 Amplite™ Red substrate stock solution (200X): Add 65 µL of DMSO (Component F) into the vial of Amplite™ Red (Component A). The stock solution should be used promptly, and any remaining solution need be aliquoted and frozen at -20 °C.
Note: Avoid repeated freeze-thaw cycles, and protect from light.

1.2 100 U/mL HRP stock solution: Add 200 µL of Assay Buffer (Component C) into the vial of Horseradish Peroxidase (Component D).
Note: The unused HRP stock solution should be divided into single use aliquots and stored at -20°C.

1.3 10 mM H_2O_2 stock solution: Add 10 µL of 3% H_2O_2 (0.88 M, Component B) into 870µL of Assay Buffer (Component C).
Note: The diluted H_2O_2 stock solution is not stable. The unused portion should be discarded.

2. Prepare H_2O_2 assay buffer:

Add 5µL of 10mM H_2O_2 stock solution (from Step 1.3) into 5mL of Assay Buffer (Component C).

3. Prepare serially diluted catalase standards (0 to2 U/mL):

Warning

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.

3.1 Add 2 µL of 1000 U/mL Catalase Standard (Component E) into 1000 µL of Assay Buffer (Component C) to get 2 U/mL catalase standard solution.

3.2 Take 500 µL of 2 U/mL catalase standard solution (from Step 3.1) to perform 1:2 serial dilutions to get 1, 0.5, 0.25, 0.125, 0.062, 0.031 and 0 U/mL serially diluted catalase standards.

3.3 Add serially diluted catalase standards and catalase-containing test samples into a solid black 96-well microplate as described in Tables 2 and 3.

Table 1. Layout of catalase standards and test samples in a solid black 96-well microplate

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

Note: CS= Catalase Standards, BL=Blank Control, TS=Test Samples.

Table 2. Reagent composition for each well

Catalase Standards	**Blank Control**	**Test Sample**
Serial dilutions*: 50 µL	Assay buffer (Component C): 50 µL	50 µL

Note: Add the serially diluted catalase standards from 0.031 U/mL to 2 U/mL into wells from PS1 to PS7 in duplicate.

4. Run catalase assay:

4.1 Add 50 µL of H_2O_2 assay buffer (from Step 2) into each well of the catalase standard, blank control, and test samples (see Step 3.3) to make the total catalase assay volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL of sample and 25 µL of H_2O_2 assay buffer into each well.

4.2 Incubate the reaction at room temperature for 15 to 30 minutes, protected from light.

4.3 Prepare assay mixture according to the following table and keep from light:

Table 3. Assay mixture for one 96-well plate

Components	Volume
Amplite™ Red substrate stock solution (200X, from Step 1.1)	25 µL
HRP stock solution (100U/mL, from Step 1.2)	15 µL
Assay Buffer (Component C)	5.0 mL

4.4 Add 50 µL of assay mixture (from Step 4.3) into each well of catalase standard, blank control, and test samples (see Step 3.3) to make the total assay volume of 150 µL/well.
Note: For a 384-well plate, add 25 µL of assay mixture into each well.

4.5 Incubate the reaction at room temperature for 15 to 30 minutes, protected from light.

4.6 Monitor the fluorescence increase at Ex/Em = 540±10/590±10 nm (optimal Ex/Em = 540/590) using a fluorescence plate reader.
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.

Data analysis

The fluorescence intensity of blank wells (no catalase, with the assay buffer only) is used as a control. For each catalase dose, the fluorescence intensity is reported as the **difference** of the observed fluorescence intensity subtracted from that of a no-catalase control. The catalase standard curve is shown in Figure 1.

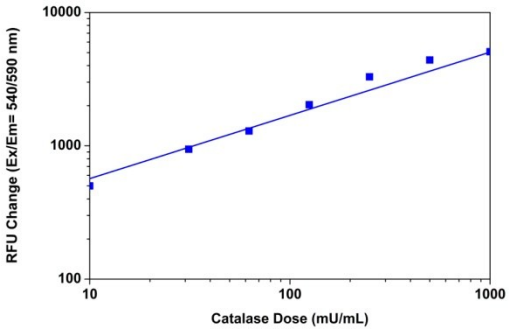

Figure 1 Catalase dose response was measured with Amplite™ Fluorimetric Catalase Assay Kit in a solid black 96-well plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 30 mU/mL catalase can be detected with 30 minutes incubation (n=3).

References

1. Porstmann, B., Porstmann, T., Nugel, E. and Evers, U. (1985). Which of the commonly used marker enzymes gives the best results in colorimetric and fluorimetric enzyme immunoassays: horseradish peroxidase, alkaline phosphatase, ß-galactosidase? *J. Immunol. Meth.* **79**, 27-37.
2. Wordinger, R.J., Miller, G.W. and Nicodemus, D.S. (1987). *Manual of Immunoperoxidase Techniques, 2nd Edition.* Chicago: American Society of Clinical Pathologists Press, pp. 23-24.
3. Yolken, R.H. (1982). Enzyme immunoassays for the detection of infectious antigens in body fluids: current limitations and future prospects. Rev. Infect. Dis. **4(1)**, 35-68.