Catalase Activity (CAT) Assay
Cat. No. 8218, 100 tests

Introduction

Catalase is an antioxidant enzyme present in most living organisms which are exposed to oxygen. It is involved in the decomposition of hydrogen peroxide (H_2O_2), a reactive oxygen species (ROS) which is a toxic product of normal aerobic metabolism. ScienCell™ Catalase Activity (CAT) Assay measures the activity of catalase based on the peroxidatic function of catalase with methanol as the hydrogen donor in the presence of H_2O_2. The formaldehyde produced is determined with purpald (4-Amino-3-hydrazino-5-mercapto-1,2,4-triazole) as a chromogen. To obtain a colored compound, the product of the reaction between formaldehyde and purpald is oxidized by potassium periodate. The absorbance can be read at 550 nm.

Kit Components

Cat. No.	# of vials	Reagent	Amount	Storage
8218a	1	CAT Assay Buffer	2.5 ml	2-8˚C
8218b	1	Methanol	2.5 ml	RT
8218c	1	10× Hydrogen Peroxide	50 µl	2-8˚, dark
8218d	1	Potassium Hydroxide	2.5 ml	2-8˚C
8218e	1	Purpald	5 ml	2-8˚C
8218f	1	Potassium Periodate	2.5 ml	2-8˚C
8218g	1	100× Catalase Standard (0.1mg/ml)	50 µl	-20˚C

Quality Control

Data from ScienCell™ CAT Assay of catalase solutions with concentrations ranging from 1 to 0.05 µg/ml shows a linear relationship between OD_{550nm} and catalase concentration (Figure 1).

Procedures

A. Preparation of catalase standards

1. Prepare a 1.0 µg/ml catalase standard by adding 10 µl of 0.1 mg/ml 100× Catalase Standard to 990 µl of DI H_2O.
2. Prepare a catalase standard curve using the serial dilutions of the 1.0 µg/ml catalase standard according to Table 1. 300 µl of catalase solution is prepared for each point to provide three replicates of 100 µl.

B. Preparation of cell lysate

1. Remove culture medium from the cultured cells, wash cells twice with ice-cold PBS and remove PBS.

2. Add 100 µl of ice-cold 1% Triton X-100 to each sample well of 24-well plate (~0.1-1×10^5 cells) and gently rock the plate side-to-side. For cells in different size wells, scale up or down the volume

of 1% Triton X-100 according to the surface area of the wells.

3. Incubate at 2-8ºC for 20 min with gentle agitation to lyse cells. Centrifuge the lysate at 14,000 × g in pre-cooled centrifuge for 3 minutes, transfer the supernatant to fresh tube and discard the pellet. Cell lysate can be stored at -70 ºC or used immediately for catalase measurement.

C. Assay procedure

1. Making working hydrogen peroxide solution by diluting appropriate volume of 10× Hydrogen Peroxide ten times with DI H_2O. Add 25 µl of CAT Assay Buffer, 25 µl of methanol and 5 µl of working hydrogen peroxide solution to each well of 96 well plate.
2. Initiate the reaction by adding 50 µl of catalase standard or sample to each well. Incubate on a shaker for 20 minutes at room temperature.
3. Terminate the reaction by adding 25 µl of Potassium Hydroxide to each well.
4. Add 50 µl of Purpald to each well and incubate for 10 minutes at room temperature.
5. Add 25 µl of Potassium periodate to each well, incubate for 5 minutes and read the absorbance at 550 nm on a plate reader.

D. Calculations

1. Average the OD_{550nm} of replicate wells of each catalase standard, sample and blank. Subtract the average OD_{550nm} value of the blank from the average OD_{550nm} values obtained with all other samples.

2. Based on the calibrated OD_{550nm} of the catalase standard, make a standard curve by plotting OD_{550nm} as a function of catalase concentration. (See Figure 1 for a typical standard curve.) Determine the equation and R^2 value of the trend line.

3. Suppose the equation of the trend line of the standard curve is $y = Ax + B$, calculate the catalase concentration of samples as follows:

$$[Catalase] = \frac{OD_{550\,nm} - B}{A}$$

No.	1 µg/ml Catalase (µL)	DI H_2O (µL)	Catalase concentration (µg/mL)
1	300	0	1
2	240	60	0.8
3	180	120	0.6
4	120	180	0.4
5	60	240	0.2
6	30	270	0.1
7	15	285	0.05
8	0	300	0 (Blank)

Table 1. Preparation of catalase standards in ScienCell™ CAT Assay.

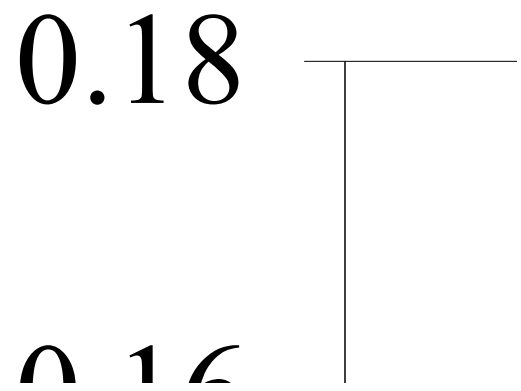

Figure 1. A typical catalase standard curve measured by ScienCell™ CAT Assay.