Catalase Activity Assay Kit

KB-03-012

100/200/500 test (96 well plate)

BQCkit

A brand of BioQuChem

Index

All chemicals should be handled with care

- This kit is for R&D use only

Introduction

Catalase is an enzyme present in blood and other tissues with antioxidant activity. This enzyme can catalyze the reaction that consumes hydrogen peroxide, transforming it into water and oxygen. Since hydrogen peroxide is difficult to be measured directly in biological samples, the determination of these detoxifying enzymes has been widely used in substitution. Catalase activity levels are also related to antioxidant capacity.

Assay Principle

Catalase enzyme performs a reaction giving rise to a compound that forms a complex with the chromogen. This reaction produces a purple color directly proportional to catalase activity that can be determined by means of a simple and fast spectrophotometrical measure.

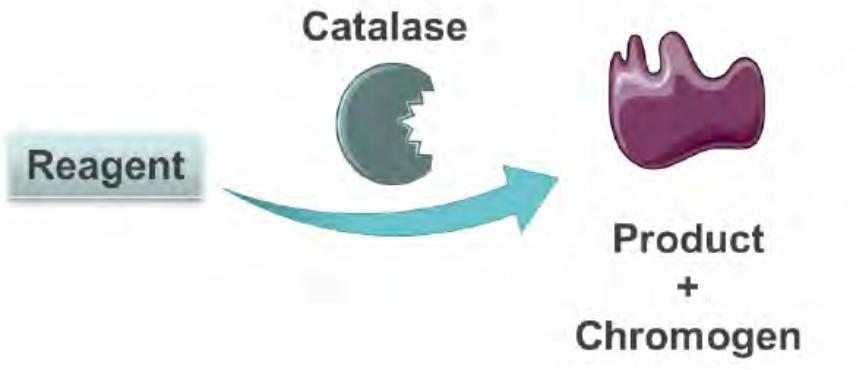

Figure 1. *Principle of the assay reaction*

Materials

BQCkit Catalase Activity Assay kit *KB-03-012 100/200/500 tests* contains:

Product	Quantity 100 tests	Quantity 200 tests	Quantity 500 tests	Storage
Standard	1 vial	2 vials	5 vials	4ºC
Reagent A	1 bottle	2 bottles	5 bottles	4ºC
Reagent B	1 bottle	2 bottles	5 bottles	4ºC
Reagent C	1 vial	2 vials	5 vials	4ºC
Reagent D	1 vial	2 vials	5 vials	4ºC
Reagent E	1 vial	2 vials	5 vials	4ºC
Reagent F	1 vial	2 vials	5 vials	4ºC
Reagent G	1 vial	2 vials	5 vials	4ºC
Reagent H	1 vial	2 vials	5 vials	4ºC

Sample Preparation

Tissue homogenate

Rinse tissue with PBS (pH 7.4).

Homogenize in 5-10 mL of cold buffer/g tissue.

Centrifuge at 10,000 x g for 15 min at 4ºC.

Collect the supernatant to assay or freeze.

Cell lysate

Centrifuge sample at 1,000-2,000 x g for 10 min at 4ºC. Do not use proteolitic enzymes.

Homogenize/ sonicate cell pellet with 1-2 mL of cold buffer.

Centrifuge at 10,000 x g for 15 min at 4ºC.

Collect the supernatant to assay or freeze.

Plasma

Centrifuge blood simple (with anticoagulant) at 700-1,000 x g for 10 min at 4ºC.

Collect the supernatant to assay or freeze.

Reagent Preparation

Standard solutions:

Dilute 10 µL of the standard solution in 9990µl of double distillated water or Milli Q water. This will be called Standard stock.

Prepare several solutions for the calibration curve with Reagent A as diluent. The first one will be the blank tube.

Table 1. *Reagent volumes needed to carry out the standard curve*

Standard concentration [µM]	Standard [µL]	Reagent A [µL]
0	0	1000
15	30	970
30	60	940
45	90	910
60	120	880
75	150	850

Positive Control:

Resuspend each vial of Reagent D in 1 mL of Reagent B (Assay Buffer). This solution is stable for at least 2 hours.

Substrate:

Dilute 40 µL of Reagent E in 9.96 mL of double distillated water or Milli Q water. This solution is called Substrate and is stable for at least 2 hours.

Assay Protocol

Short protocol:

This protocol can be performed both, in 1.5ml tubes or in 96 well plate directly. This short protocol is for 1.5 ml tubes (this protocol is better due to the lack of bubbles). For 96 well plate directly analysis (high presence of bubbles), replace 1.5 ml tubes for 96 well plate in points 1 to 4.

1 

In 1.5 ml tubes (not included) add:
Standard/sample/positive control: 20 µL

2 

Then, add to each tube:
Reagent B: 100 µL
Reagent C:30 µL
Substrate (previously prepared): 20µL

3 

Let the reaction run for 20 minutes.

4 

Then, add to each tube:
Reagent F: 30 µL

5 

Carefully, transfer the content of each tube (200ul) to each of the wells of the 96 well plate.

6 

Immediately after, add 30 µL of the Reagent G (Chromogen Solution) to each well.

7 

Let the reaction run for 10 minutes.

8 

Add 20 µL of Reagent H to each well.

9 

Let the reaction run for 5 minutes and then read the absorbance at 540 nm.

Assay Protocol

Detailed protocol:

Preparing the tubes

Each sample and the standard should be done at least duplicated. First of all:

- Standard tubes: Each tube should contain 20 µL of the correspondent standard (previously prepared- see Standard Solutions). The first standard solution will be the blank of the assay.
- Sample tubes: Add 20 µL of sample.
- Positive control: Add 20 µL of Positive control previously prepared (see Reagents Preparation).

Then add to each tube:

- 100 µL of the Reagent B (Assay buffer) to each tube avoiding the formation of bubbles.
- 30 µL of Reagent C.

Start the reaction:

Add 20 µL of Substrate previously prepared (see Reagents Preparation) to all the tubes. Shake smoothly the tubes since the addition of Substrate reagent.

Stop the reaction

Add 30 µL of the Reagent F (Stop Solution).

Assay Protocol

Chromogen Reaction

Carefully, transfer the content of each tube (200ul) to each of the wells of the 96 well plate.

Add 30 µL of the Reagent G (Chromogen Solution) immediately after. The wells should start having a slightly purple colour. Let the reaction run for 10 minutes and shake smoothly with hands at minute 5 since the addition of the chromogen.

After 10 minutes, add 20 µL of the Reagent H. Wait for 5 minutes. Shake the plate smoothly for 1 minute.

Measurement

Place the plate in the reader and measure the absorbance at 540 nm.

Data Analysis

Analysis of the Standard: Represent the absorbance against the concentration of the standard, including the equation and the R^2. Subtract the absorbance obtained for the standard well A (the blank) to all absorbances obtained, including this one.

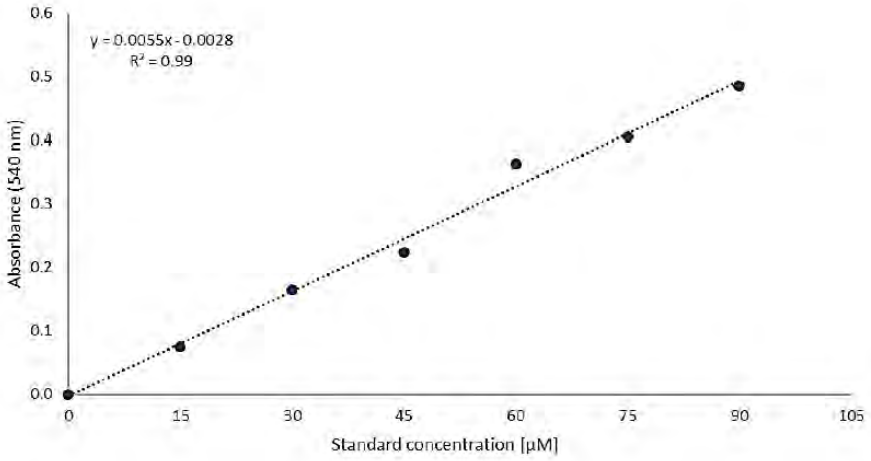

Figure 1. *Example of the standard representation*

Analysis of the Catalase samples: The equation obtained from the standard can be used to obtain the concentration of the substance produced by the enzyme. The equation should be like this, in which “y” represents the absorbance and “x” the concentration of the product in µM.

$$Y = aX - b$$

$$X = [(y\text{-intercept})/slope] \times (0.17\ mL / 0.02\ mL)$$

Data Analysis

The catalase activity can be determined with this formula:

CAT activity= (μM of the product in the sample/20 min) x sample dilution = (nmol/(mL x min)) = mU/mL

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