Catalase Activity Assay

(Cat. # BAQ061, BAQ062, BAQ063)

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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.

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.

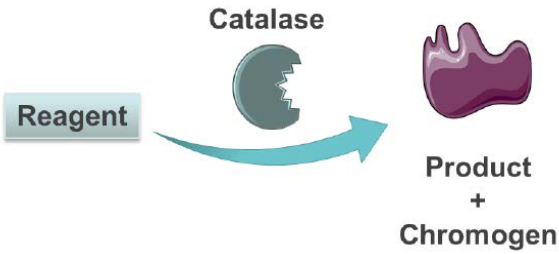


Figure 1. ***Principle of the assay reaction***

ITEM(S) SUPPLIED

Description	**Cat # BAQ061 100 tests (96 well plate)**	**Cat # BAQ062 200 tests (96 well plate)**	**Cat # BAQ063 500 tests (96 well plate)**
CAA Standard	1 vial	2 vials	5 vials
CAA Reagent A (Standard Diluent)	1 bottle	2 bottles	5 bottles
CAA Reagent B (Assay Buffer)	1 bottle	2 bottles	5 bottles
CAA Reagent C	1 vial	2 vials	5 vials
CAA Reagent D (Positive Control)	1 vial	2 vials	5 vials
CAA Reagent E (Substrate)	1 vial	2 vials	5 vials
CAA Reagent F	1 vial	2 vials	5 vials
CAA Reagent G (Chromogen)	1 vial	2 vials	5 vials
CAA Reagent H	1 vial	2 vials	5 vials

STORAGE CONDITIONS

The kit is supplied on blue ice. Store all reagents at 4°C upon arrival. If stored and used as directed this kit is stable for 12 months.

ADDITIONAL ITEMS REQUIRED

- Spectrophotometer microplate reader that can measure at 540 nm
- 96 well microtiter plate for microplate assay.
- 1.5ml Tubes

SAMPLE PREPARATION

Tissue Homogenate

1. Rinse tissue with PBS
2. Homogenize in 5-10ml cold buffer for every gram of tissue
3. Centrifuge at 10,000xg for 15 minutes at 4°C
4. Collect the supernatant to assay or freeze at -80°C

Cell Lysate

1. Collect the cells by centrifugation at 1,000-2,000xg for 10 minutes at 4°C
2. Homogenize of sonicate the cell pellet with 1-2ml cold buffer
3. Centrifuge at 10,000xg for 15 minutes at 4°C
4. Collect the supernatant to assay or freeze at -80°C

Plasma

1. Centrifuge blood with an anticoagulant at 700-1,000xg for 10 minutes at 4°C
2. Collect the supernatant to assay or freeze at -80°C

PREPARATION BEFORE USE

Standard solutions

1. Dilute 10 µL of the standard solution in 9990µl of double distilled water or Milli Q water. This is the **Standard stock.**
2. Prepare several solutions for the calibration curve with **CAA Reagent A** as diluent. The first one will be the blank tube.

Standard concentration [µM]	Standard Stock [µL]	CAA 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 **CAA Reagent D** in 1 mL of **CAA Reagent B (Assay Buffer)**. This solution is stable for at least 2 hours.

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

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 preferred due to the lack of bubbles). For 96 well plate, replace 1.5 ml tubes for 96 well plate in points 1 to 4.

1. In 1.5 ml tubes (not included), add 20µl Standard/sample/positive control
2. Next add 100µl CAA Reagent B, 30µl CAA Reagent C and then 20µl previously prepared Substrate.
3. Incubate the reaction for 20 minutes.
4. Add 30µl CAA Reagent F to each tube:
5. Carefully, transfer the content of each tube (200µl) to each of the wells of the 96 well plate.
6. Immediately, add 30µl CAA Reagent G (Chromogen Solution) to each well.
7. Incubate the reaction for 10 minutes.
8. Add 20µl of Reagent H to each well.
9. Incubate the reaction for 5 minutes and then read the absorbance at 540 nm.

Detailed Protocol

Preparing the tubes

Each sample and the standard should be done at least duplicated. Prepare the following tubes:

- **Standard tubes**: Each tube should contain 20µl of the corresponding standard. The first standard solution will be the blank of the assay.
- **Sample tubes:** Add 20µl of sample.
- **Positive control:** Add 20µl of Positive control previously prepared

Next add 100µl of the CAA Reagent B (Assay buffer) and 30µl CAA Reagent C to each tube avoiding the formation of bubbles.

Start the reaction

Add 20µl of Substrate previously prepared to all the tubes. Gently mix the tubes.

Stop the reaction

Add 30µl of the CAA Reagent F (Stop Solution).

Chromogen Reaction

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

Immediately, add 30µl of the CAA Reagent G (Chromogen Solution). The wells should begin changing to a slightly purple color. Let the reaction run for 10 minutes and gently shake the plate 5 minutes after the addition of the chromogen.

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

Measurement

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

DATA ANALYSIS

Analysis of the Standard: Plot 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.

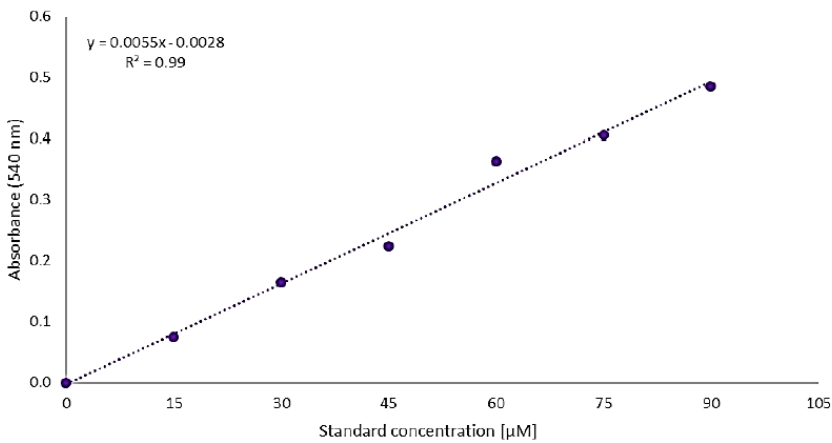


Figure 1. Example of the standard representation

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.

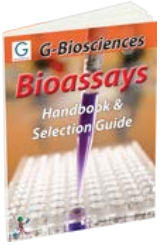
Calculation

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

RELATED PRODUCTS

Download our Bioassays Handbook.



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