CATALASE ACTIVITY
ASSAY KIT

*KB-03-012*

*100 test (96 well plate)*
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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.
Materials

Bioquochem Catalase Activity Assay Kit (KB-03-012) contains:

<table>
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<th>Product</th>
<th>Quantity</th>
<th>Storage</th>
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<tbody>
<tr>
<td>Standard</td>
<td>1 vial</td>
<td>4 ºC</td>
</tr>
<tr>
<td>Reagent A (Standard Buffer)</td>
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</tr>
<tr>
<td>Reagent B (Wells Buffer)</td>
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</tr>
<tr>
<td>Reagent C</td>
<td>1 vial</td>
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</tr>
<tr>
<td>Reagent D (Positive control)</td>
<td>1 vial</td>
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</tr>
<tr>
<td>Reagent E</td>
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</tr>
<tr>
<td>Reagent F (Stop solution)</td>
<td>1 vial</td>
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</tr>
<tr>
<td>Reagent G (Chromogen solution)</td>
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</tr>
<tr>
<td>Reagent H</td>
<td>1 vial</td>
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➢ This kit is for R&D use only

⚠️ All these chemicals should be handled with care
**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.*
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 4°C
Collect the supernatant to assay or freeze.

Cell lysate

Centrifuge sample at 1,000-2,000 x g for 10 min at 4°C. Do not use proteolytic enzymes.
Homogenize/sonicate cell pellet with 1-2 ml of cold buffer.
Centrifuge at 10,000 x g for 15 min at 4°C
Collect the supernatant to assay or freeze.

Plasma

Centrifuge blood sample (with anticoagulant) at 700-1,000 x g for 10 min at 4°C.
Remove the top yellow layer. Collect plasma to assay or freeze.
Sample preparation

Erythrocyte lysate

Centrifuge blood sample (with anticoagulant) at 700-1,000 x g for 10 min at 4°C.

Remove the top yellow layer and white layer.

Lyse with HPLC-water. The amount of water should be four times the sample amount.

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

Collect the supernatant to assay (store on ice) or freeze.

Serum

Do not use anticoagulant. Let the blood clot for 30 minutes at RT.

Centrifuge at 2,000 x g for 15 min at 4°C.

Collect the yellow layer of the supernatant to assay (store on ice) or freeze.
Reagents preparation

Standard solutions:
Dilute 10 μl of the standard solution in 9.99 ml of double distillated water or Milli Q water. This will be the standard stock.

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

<table>
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<tr>
<td>90</td>
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<td>820</td>
</tr>
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Reagent D:
Resuspend Reagent D in 1 ml of Wells Buffer (Reagent B). This solution is stable for at least 2 hours.

Reagent E:
Dilute 40 μl of reagent E in 9.96 ml of double distillated water or Milli Q water. This solution is stable for at least 2 hours.
Assay Protocol

Short protocol:

1. Add 100 µl of Reagent B and 30 µl of Reagent C to every well.

Add the following reagents:

**STANDARD:** Add 20 µl of Standard.

**SAMPLE:** Add 20 µl of sample.

**POSITIVE CONTROL:** Add 20 µl of Reagent D

2. Add 20 µl of Reagent E solution.

3. Let the reaction run for 20 minutes

4. Add 30 µl of the Stop solution (Reagent F) to all wells.

   Immediately after add 30 µl of the chromogen solution (Reagent G)

5. Let the reaction run for 10 minutes

6. Add 20 µl of Reagent H

7. Let the reaction run for 5 minutes

8. Read the absorbance at 540 nm
Assay Protocol

Detailed protocol:

Preparing the plate
Design the plate distribution of the samples in the plate. Standard can be done in a separately plate or together with the catalase samples. Each sample and the standard should be done at least duplicated. First of all:

- Add 100 μl of the Wells buffer (Reagent B) to every well used in the assay avoiding the formation of bubbles. If any bubble appears, remove it with a clean pipette tip.
- Add 30 μl of Reagent C to every well used in the assay. Avoid the use of markers at this point, is preferable to record the design of the plate in a separate sheet.

Then, depending on the type of well it should contain:

- Standard wells: Each well should contain 20 μl of the correspondent diluted standard. The first standard solution will be the blank of the assay.
- Sample wells: Add 20 μl of sample.
- Positive control: Add 20 μl of Reagent D previously prepared (see Reagents Preparation).

Start the reaction:
Add 20 μl of Reagent E previously diluted (see Reagents Preparation) to all the wells used and let it run for exactly 20 minutes. Shake the plate smoothly with hands every 5 minutes since the addition of Reagent E.
Assay Protocol

Stop the reaction
Add 30 μl of the stop solution (Reagent F) and other 30 μl of the chromogen solution (Reagent G) 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.

![Graph of absorbance against concentration](image)

**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.

\[ Y = aX - b \]

\[ X = \frac{(y - \text{intercept})}{\text{slope}} \times \frac{0.17 \text{ ml}}{0.02 \text{ ml}} \]

The catalase activity can be determined with this formula:

\[ \text{CAT activity} = \left( \frac{\mu M \text{ of the product in the sample}}{20 \text{ min}} \right) \times \text{sample dilution} = \left( \frac{\text{nmol}}{\text{ml} \times \text{min}} \right) = \text{mU/ml} \]
Warranties and Limitation of Liability

Bioquochem shall not in any event be liable for incidental, consequential or special damages of any kind resulting from any use or failure of the products, even if Bioquochem has been advised of the possibility of such damage including, without limitation, liability for loss of use, loss of work in progress, down time, loss of revenue or profits, failure to realize savings, loss of products of buyer or other use or any liability of buyer to a third party on account of such loss, or for any labor or any other expense, damage or loss occasioned by such product including personal injury or property damage is caused by Bioquochem’s gross negligence. Any and all liability of Bioquochem hereunder shall be limited to the amounts paid by buyer for product.

Buyer’s exclusive remedy and Bioquochem’s sole liability hereunder shall be limited to a refund of the purchase price, or the replacement of all material that does not meet our specifications.

Said refund or replacement is conditioned on buyer giving written notice to Bioquochem within 30 days after arrival of the material at its destination.

Expiration date: 1 year from the date of delivery

For further details, please refer to our website www.bqckit.com.