Dynamic Test Kits for R&D and Quality Control

CATALASE ACTIVITY ASSAY KIT

KB03012

100/200/500 TESTS

96 well plate

BQCkit

A brand of Bio QuaChem

Votre interlocuteur en France, Belgique, Luxembourg et Suisse :
LIBIOS
83, rue Edmond Michelet - 69490 Pontcharra Sur Turdine - France
Tél. : +33 (0)4 74 13 03 02 - Fax : +33 (0)4 74 05 28 25
Mail : info@libios.fr - www.libios.fr

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1. GENERAL INFORMATION

Please read this manual carefully before performing the assay.

PRECAUTIONS

This product is designed for research use only, it is not approved for human or animal use, or clinical diagnosis.

All chemicals should be handled with care and in accordance with laboratory safety practices. Maintain order and cleanliness where dangerous products are used. It is recommended to use basic Personal Protective Equipment. For more information on the risks and preventative measures, check the MSDS available at www.bqckit.com.

Do not use after the expiring date. Store reagents as indicated on the section Materials on page 5.

TECHNICAL RECOMMENDATIONS

Keep enzymes, heat labile components and samples on ice. Let the components reach room temperature before use.

Invert the bottles a few times to ensure the reagents are well mixed before running the assay. Avoid foaming or bubbles when mixing or reconstituting components. Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions. Ensure plates are properly sealed or covered during incubation steps. Ensure complete removal of all solutions and buffers from tubes or plates during wash steps. Make sure you have the right type of plate for your detection method of choice.

Make sure the heat block/water bath and microplate reader are switched on.

Do not run the standard curve and the samples at different times and do not reuse the calculations of another day. Keep the standard and the samples on the assay for the same amount of time. It is recommended to use a multi-channel pipette if possible.

2. TECHNICAL SPECIFICATIONS

Available sizes:

100 tests: 6 standard, 44 samples

200 tests: 6 standard, 94 samples

500 tests: 6 standard, 244 samples

The calculations are just an estimation assuming that all the samples were tested the same day and that every standard and sample is tested on duplicate. Test number refers to total number of wells to be evaluated.

Volume of sample required:

20 µl/test

Types of sample compatible:

Plasma, serum, tissue, homogenates and cell lysates.

Linear range:

6 – 30 U/ml

Type of detection:

Colorimetric (540 nm)

Sensitivity:

0.03 OD 540 nm/(U/ml)

Time required for the assay:

60 min

3. MATERIALS

MATERIALS SUPPLIED

Store kit components as indicated below:

100 tests

Product	Nº bottles	Amount	Storage (before use)	Storage (after use)
Reagent A	1 bottle	20 ml	4ºC	4ºC
Reagent B	1 bottle	20 ml	4ºC	4ºC
Reagent C	1 vial	4 ml	4ºC	4ºC
Reagent D	1 vial	Powder	4ºC	-
Reagent E	1 vial	4 ml	4ºC	-
Reagent F	1 vial	4 ml	4ºC	4ºC
Reagent G	1 vial	3.3 ml	4ºC	4ºC
Reagent H	1 vial	2.5 ml	4ºC	4ºC
Standard	1 vial	500 µl	4ºC	4ºC
96-well plate	-	1	-	-

200 tests

Product	Nº bottles	Amount	Storage (before use)	Storage (after use)
Reagent A	1 bottle	40 ml	4ºC	4ºC
Reagent B	1 bottle	40 ml	4ºC	4ºC
Reagent C	1 bottle	8 ml	4ºC	4ºC
Reagent D	1 vial	Powder	4ºC	-20ºC
Reagent E	1 bottle	8 ml	4ºC	-
Reagent F	1 bottle	8 ml	4ºC	4ºC
Reagent G	2 vials	3.3 ml	4ºC	4ºC
Reagent H	1 vial	5 ml	4ºC	4ºC
Standard	1 vial	1 ml	4ºC	4ºC
96-well plate	-	1	-	-

500 tests

Product	Nº bottles	Amount	Storage (before use)	Storage (after use)
Reagent A	1 bottle	100 ml	4ºC	4ºC
Reagent B	1 bottle	100 ml	4ºC	4ºC
Reagent C	1 bottle	20 ml	4ºC	4ºC
Reagent D	1 vials	Powder	4ºC	-20ºC
Reagent E	1 bottle	20 ml	4ºC	-
Reagent F	1 bottle	20 ml	4ºC	4ºC
Reagent G	5 vials	4 ml	4ºC	4ºC
Reagent H	3 vials	5 ml	4ºC	4ºC
Standard	1 vials	2.5 ml	4ºC	4ºC
96-well plate	-	1	-	-

MATERIALS NEEDED BUT NOT SUPPLIED

Materials:

- Double distilled water (ddH2O) as MilliQ
- Pipettes and pipette tips
- 1.5 ml tubes

Instrumentation:

- Microcentrifuge
- Vortex mixer
- Colorimetric microplate reader – equipped with filter for OD 540 nm

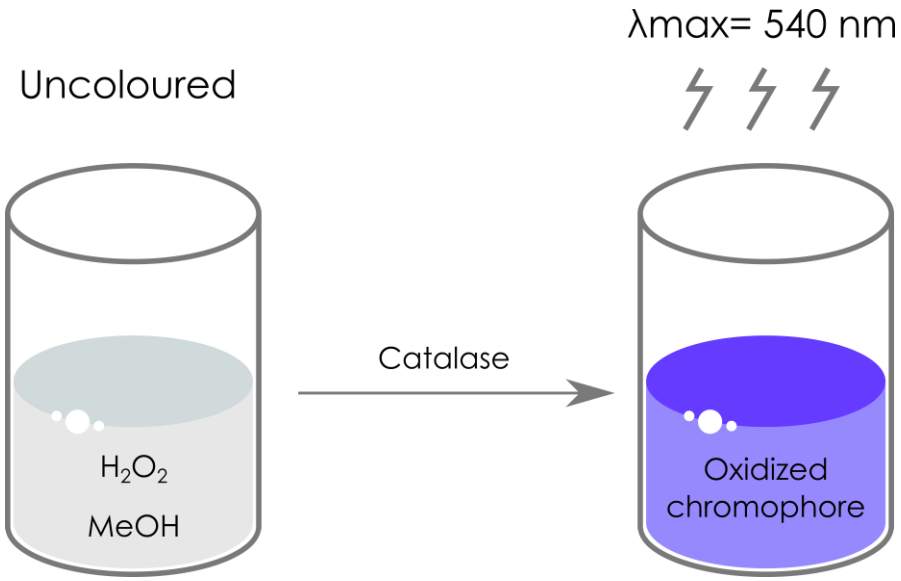
4. INTRODUCTION

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.

BQC Catalase Activity Kit is a highly sensitive and reproducible assay to test catalase activity in biological samples as plasma, tissue homogenates and cell lysates.

5. ASSAY PRINCIPLE

This kit measures the catalase activity of biological samples. Catalase enzyme performs a reaction in which hydrogen peroxide and methanol are involved. In this reaction, formaldehyde is produced and reacts with the chromogen, oxidizing it, which shows a maximum of absorbance at 540 nm. This reaction is directly proportional to catalase activity.



6. SAMPLE PREPARATION

BQCkit have tested the samples indicated below.

Sample	Preparation required	Dilution factor	Diluent	Long term storage
Plasma	No	-	-	-20ºC

Samples from abnormal or extreme experimental conditions may require a different dilution factor.

Is your sample is not included on this list? Check the BQCkit Testing Program and get a discount on your next order!

POSITIVE CONTROL

BQCkit adds a catalase containing vial to make it possible to run a positive control. Using the positive control is completely optional and is not required for the correct performance of the assay.

PREPARATION PROTOCOLS

Reagents required for sample preparation are not supplied. Take in account the sample volume required per test, refer to section Technical Specifications on page 4.

Tissue homogenate:

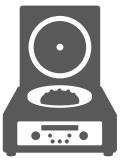
1

Rinse tissue with PBS (pH 7.4)

2

Homogenize in 5-10 ml of cold buffer/g tissue

3



Centrifuge at 10,000 xg for 15 min at 4 ºC

4

Collect supernatant to assay or freeze

Total time required: 30 min

Cell lysate:

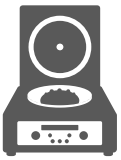
1

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

2

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

3



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

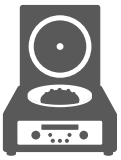
4

Collect supernatant to assay or freeze

Total time required: 35 min

Plasma:

1



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

2

Collect the supernatant to assay or freeze

Total time required: 20 min

7. ASSAY PREPARATION

REAGENT PREPARATION

Reagents not included on this list are ready to use as supplied.

- Positive control: Resuspend each vial of Reagent D that is going to be used immediately with 1 ml of Reagent B. This solution is stable for at least 2 hours at room temperature, to use at a different time, store at -20ºC.
- Solution E: Add 40 µl of Reagent E to 9.96 ml of ddH_2O. This solution is stable for at least 2 hours, discard remaining solution E after the assay.

STANDARD PREPARATION

Add exactly 10 µl of the Standard to 9990 µl of ddH_2O to obtain a 1:100 dilution of the Standard. Use this diluted standard solution to prepare the calibration curve.

Prepare the calibration curve in 1 mL tubes as shown below. Use Reagent A as the diluent.

	Standard (µl)	Reagent A (µl)	Enzymatic activity (U/ml)
1	0	1000	0
2	30	970	6.375
3	60	940	12.75
4	90	910	19.125
5	120	880	25.5
6	150	850	31.875

The standard is expressed as enzymatic activity of the catalase. A unit is defined as the amount of enzyme that converts 1 µmol of substrate to product per minute of reaction.

PLATE SET UP

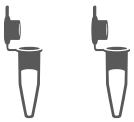
This scheme is just a recommendation on how to perform the assay. For optimal results, BQCkit recommends running the standards and the samples at least for duplicate, but it is the user´s discretion to do so.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	C2	C2	C10	C10	C18	C18	C26	C26	C34	C34
B	S2	S2	C3	C3	C11	C11	C19	C19	C27	C27	C35	C35
C	S3	S3	C4	C4	C12	C12	C20	C20	C28	C28	C36	C36
D	S4	S4	C5	C5	C13	C13	C21	C21	C29	C29	C37	C37
E	S5	S5	C6	C6	C14	C14	C22	C22	C30	C30	C38	C38
F	S6	S6	C7	C7	C15	C15	C23	C23	C31	C31	C39	C39
G	PC	PC	C8	C8	C16	C16	C24	C24	C32	C32	C40	C40
H	C1	C1	C9	C9	C17	C17	C25	C25	C33	C33	C41	C41

S1-S6: Standard wells, PC: Positive control, C1-C41: Sample wells

8. ASSAY PROTOCOL

Before performing the assay, check the section Technical recommendations on page 3 to avoid any mistakes. To perform a duplicate, two separate 1.5 ml tubes should be used for each sample, positive control and standard point.

1 

In 1.5 ml tubes (not included) add 20 µl of the sample, positive control or standard previously prepared (refer to sections Sample preparation on page 9 and Standard preparation on page 11).

2 

Add 100 µl of Reagent B in each sample, positive control and standard tube. Avoid the formation of bubbles.

3 

Add 30 µl of Reagent C in each sample, positive control and standard tube.

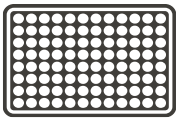
4 

Add 20 µl of Solution E previously prepared (refer to section Reagent preparation on page 11) in each sample, positive control and standard tube to initiate the reaction.

5 

Mix with a vortex mixer and let the reaction run for 20 minutes.

6  Add 30 µl of Reagent F in each sample, positive control and standard tube to stop the reaction.

7  Set up the plate design, you can use the BQCkit recommended set up (refer to section Plate set up on page 12) or use your own (refer to section Researcher notes on page 19)

8  Carefully, transfer 200 µl of each tube content to the correspondent well of the 96 well plate.

9  Add 30 µl of Reagent G in each sample, positive control and standard well. The wells should start having a slightly purple colour.

10  Let the reaction run for 10 minutes

11  Add 20 µl of Reagent H in each sample, positive control and standard well.

12  Let the reaction run for 5 minutes.

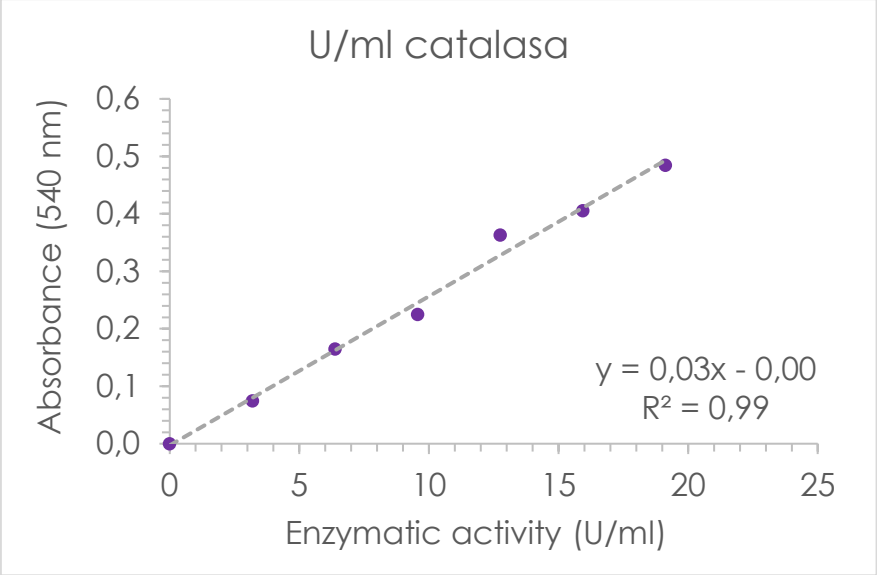
13  Shake the plate smoothly for 1 minute and read the absorbance at 540 nm.

9. DATA ANALYSIS

ANALYSIS OF THE STANDARD

If the spectrophotometer or microplate reader was not zeroed with the blank, then average the blank values and subtract the average blank value from the standard and unknown sample values.

Create a standard curve by plotting the absorbance at 540 nm (y-axis) vs. standard enzymatic activity (U/ml) (x-axis).



ANALYSIS OF THE SAMPLE

Determine the unknown sample concentration using the standard curve from the assayed sample value, and then apply:

Enzymatic activity (U/ml) =

(OD 540 nm-intercept/slope)*dilution factor)

10. INTERFERING SUBSTANCES

The following substances have been found to interfere with the assay:

- Catalase inhibitors: 3 Amino 1,2,4, triazole and 2,4 dichlorophenol.

11. TROUBLE SHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of the instrument
	Use of a different 96 well-plate	Colorimetric: Clear plates, Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use TCA precipitation protocol for deproteinization
	Cells/Tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at 80ºC (after snap freeze in liquid nitrogen) till use
	Presence of interfering substances in the sample	Check protocol for interfering substances
Lower/Higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use

	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (<5 µl) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/below the linear range	Concentrate/Dilute sample so it is within the linear range

12. RESEARCHER NOTES

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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G												
H												

13. WARRANTIES AND LIMITATION OF LIABILITY

Our partner 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, downtime, 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 the buyer for the 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 the arrival of the material at its destination.

Expiration date: 1 year from the date of delivery

Votre interlocuteur en France, Belgique, Luxembourg et Suisse :
LIBIOS
83, rue Edmond Michelet - 69490 Pontcharra Sur Turdine - France
Tél. : +33 (0)4 74 13 03 02 - Fax : +33 (0)4 74 05 28 25
Mail : info@libios.fr - www.libios.fr