XANTHINE OXIDASE ASSAY KIT

KB03032

100/200/400 TESTS

96 well plate

BQCkit

A brand of Bio QuChem

INDEX

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 bqckit.com.

Do not use after the expiring date. Store reagents as indicated on the section **Materials** on page 6.

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

400 tests: 6 standard, 194 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:

10 µl/test

Types of sample compatible:

Biological fluids, tissue homogenates and dairy products

Linear range:

0.02 – 0.8 U/ml

Type of detection:

Colorimetric (290 nm)

Sensitivity:

0.0352 U/ml/Slope OD 290 nm 20 min (especificar unidades para cada kit)

Time required for the assay:

30 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	5 ml	4 °C	4 °C
Reagent B	1	21 ml	4 °C	4 °C
Standard	1	8 µl	4 °C	4 °C*
96-well plate	1	-	-	-

Once diluted, each vial of Standard remains stable at 4 °C for at least 3 months.

200 tests

Product	Nº bottles	Amount	Storage (before use)	Storage (after use)
Reagent A	1	10 ml	4 °C	4 °C
Reagent B	1	41 ml	4 °C	4 °C
Standard	2	8 µl	4 °C	4 °C*
96-well plate	2	-	-	-

Once diluted, each vial of Standard remains stable at 4 °C for at least 3 months.

400 tests

Product	N° bottles	Amount	Storage (before use)	Storage (after use)
Reagent A	1	20 ml	4 °C	4 °C
Reagent B	1	81 ml	4 °C	4 °C
Standard	4	8 µl	4 °C	4 °C*
96-well plate	3	-	-	-

Once diluted, each vial of Standard remains stable at 4 °C for at least 3 months.

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 290 nm

4. INTRODUCTION

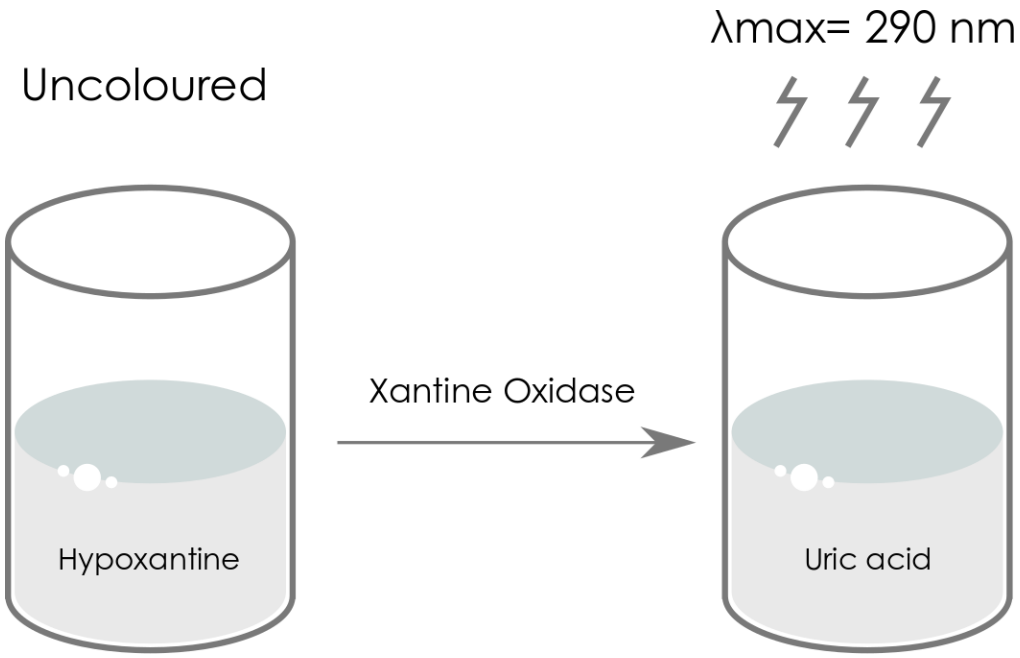
Xanthine oxidase is a molybdoflavoprotein enzyme, present in various tissues and biological fluids like liver, lungs, heart, vascular endothelium, lactating mammary gland and milk. In mammals, it plays an important role in the metabolism of purines.

Its activity produces reactive oxygen species like superoxide and hydrogen peroxide and is associated with oxidative stress in multiple pathologies and conditions like ischemia-reperfussion, hyperglycemia, hypercholesterolemia, hiperlipidemia, cardiovascular disease and liver disease.

BQC Xanthine oxidase activity assay kit is a quick, very simple and one-step test, with high reproducibility and low detection limits to assay the activity of xanthine oxidase in various samples.

5. ASSAY PRINCIPLE

Xanthine oxidase catalyzes a two step reaction that converts hypoxanthine to xanthine and then xanthine to uric acid. By doing so, it produces hydrogen peroxide and superoxide. The production of uric acid can be monitored at 290 nm. The rate of the production of uric acid is proportional to the activity of the xanthine oxidase.



6. SAMPLE PREPARATION

BQCkit have tested the samples indicated below.

Sample	Preparation required	Dilution factor	Diluent	Long term storage
Plasma	Yes	-	-	-80 °C

Samples from abnormal or extreme experimental conditions may require a different dilution factor. For sample preparation instructions refer to the section **Preparation protocols** on page 10.

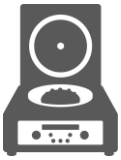
Is your sample is not included on this list? Check the BQCkit Testing Program and get a discount on your next order!

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 5.

Plasma:

1



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

2

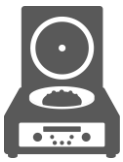
Collect the supernatant to assay or freeze

3

Concentrate with 10 kDa amicons following manufacturer instructions

Total time required: 20 min

Tissue homogenate:

1	2	3	4
			
Rinse tissue with PBS (pH 7.4)	Homogenize in 5-10 ml of cold buffer (containing protease inhibitors)/g tissue	Centrifuge at 10,000 xg for 15 min at 4 °C	Collect supernatant to assay or freeze up to 1 month

Total time required: 30 min

7. ASSAY PREPARATION

REAGENT PREPARATION

All reagents are ready to use as supplied.

STANDARD PREPARATION

Thaw vial on ice. Vortex gently and spin briefly to ensure contents are in the bottom of the vial. Add exactly 190 µl of Reagent A to the standard vials that are going to be used immediately and mix well.

Prepare the calibration curve in 1 mL tubes as shown below.

	Standard (µl)	Reagent A (µl)	Concentration (U/ml)
1	0	100	0
2	5	95	0.05
3	10	90	0.1
4	20	80	0.2
5	40	60	0.4
6	80	20	0.8

One unit of xanthine oxidase is defined as the amount of enzyme that will catalyze the conversion of one µmol of xanthine to uric acid per minute at pH 7.8 at 25 °C.

PLATE SET UP

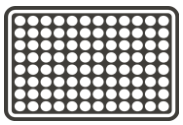
This scheme is just a recommendation on how to perform the assay. For optimal results, **BQCkit recommends running the standards and the samples at least for duplicate**, but it is the user´s discretion to do so.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	C3	C3	C11	C11	C19	C19	C27	C27	C35	C35
B	S2	S2	C4	C4	C12	C12	C20	C20	C28	C28	C36	C36
C	S3	S3	C5	C5	C13	C13	C21	C21	C29	C29	C37	C37
D	S4	S4	C6	C6	C14	C14	C22	C22	C30	C30	C38	C38
E	S5	S5	C7	C7	C15	C15	C23	C23	C31	C31	C39	C39
F	S6	S6	C8	C8	C16	C16	C24	C24	C32	C32	C40	C40
G	C1	C1	C9	C9	C17	C17	C25	C25	C33	C33	C41	C41
H	C2	C2	C10	C10	C18	C18	C26	C26	C34	C34	C42	C42

S1-S6: Standard wells, C1-C42: Sample wells

8. ASSAY PROTOCOL

Before performing the assay, check the section **Technical recommendations** on page 3 to avoid any mistakes.

1 

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 22)

2 

Add 10 µl of the sample or standard previously prepared (refer to sections **Sample preparation** on page 10 and **Standard preparation** on page 12)

3 

Add 200 µl of Reagent in each sample and standard well.

4 

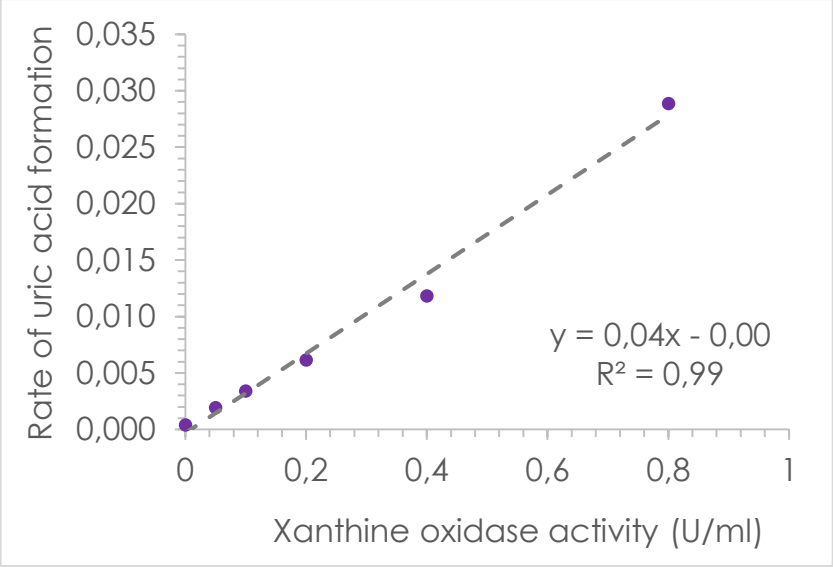
Start a kinetic measurement at an absorbance of 290 nm for 20 minutes in intervals of 1 minute.

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.

Calculate the rate of uric acid production as the slope of the absorbance at 290 nm vs time. Create a standard curve by plotting the rate of uric acid production (y-axis) vs. standard activity U/ml xanthine oxidase (x-axis).



ANALYSIS OF THE SAMPLE

Determine the unknown sample concentration using the standard curve from the assayed sample value. Average the slope for the replicates and then apply:

$$\text{Value} = \left(\frac{\text{Rate of uric acid production-intercept}}{\text{slope}}\right) * \text{dilution factor}$$

10. INTERFERING SUBSTANCES

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

- Tween 20
- Ethanol, methanol and DMSO at concentrations above 25 %

11. TROUBLESHOOTING

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 80°C (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

STILL HAVING PROBLEMS?

Please, contact BQCkit if you have any further questions, our team will be pleased to help you:

	Phone	+34 985 269 292 / 985 980 098
	E-mail	info@bioquochem.com
	Business hours	Monday-Friday: 8am to 7pm (CEST)

12. RELATED PRODUCTS

More products available on **bioquochem.com**

Reference	Product
KB03011	Superoxide Dismutase, SOD Activity Assay Kit
KB03012	Catalase Activity Assay Kit
KP06002	DHE probe (Intracellular ROS assay)
KP06003	DCFH-DA probe (Intracellular ROS assay)
KP06004	DHR-123 (Dihidrorhodamine)
KP06005	Multiprobe REDOX Assay Kit

13. REFERENCES

Are you publishing on scientific journals? Discover the BQC Reward For Citations Program and get a free kit!

14. RESEARCHER NOTES

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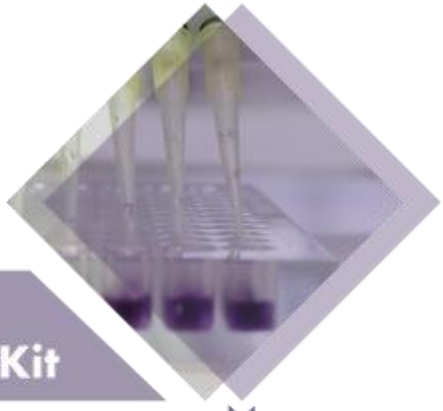
Bioquochem shall not in any event be liable for incidental, consequential or special damages of any kind resulting from any use or failure of the products, even if Bioquochem has been advised of the possibility of such damage including, without limitation, liability for loss of use, loss of work in progress, 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

For further details, please refer to our website **BQCkit**.



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