LIBIOS

Dynamic Test Kits for R&D and Quality Control

CUPRAC ASSAY KIT

KF01005

250 TESTS

96 well plate

BQCkit

A brand of BioQuoChem

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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 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:

250 tests: 6 standard, 119 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:

40 µl/test

Types of sample compatible:

Biological samples

Linear range:

0 – 2 mM

Type of detection:

Colorimetric (450 nm)

Sensitivity:

0.97 OD 450 nm/ mM Trolox

Time required for the assay:

45 min

3. MATERIALS

MATERIALS SUPPLIED

Store kit components as indicated below:

Product	Nº bottles	Amount	Storage (before use)	Storage (after use)
Reagent A	3	3.5 ml (each)	4 ºC	4 ºC
Reagent B	2	Powder	4 ºC	-
Reagent C	1	30 ml	4 ºC	4 ºC
Reagent D	1	30 ml	4 ºC	4 ºC
Reagent E	1	15 ml	4 ºC	4 ºC
Standard	2	Powder	4 ºC	-
96-well plate	-	-	-	-

Each vial of Reagent B is valid for 125 tests, discard the remaining solution after usage.

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 equipped with filter for OD 450 nm

4. INTRODUCTION

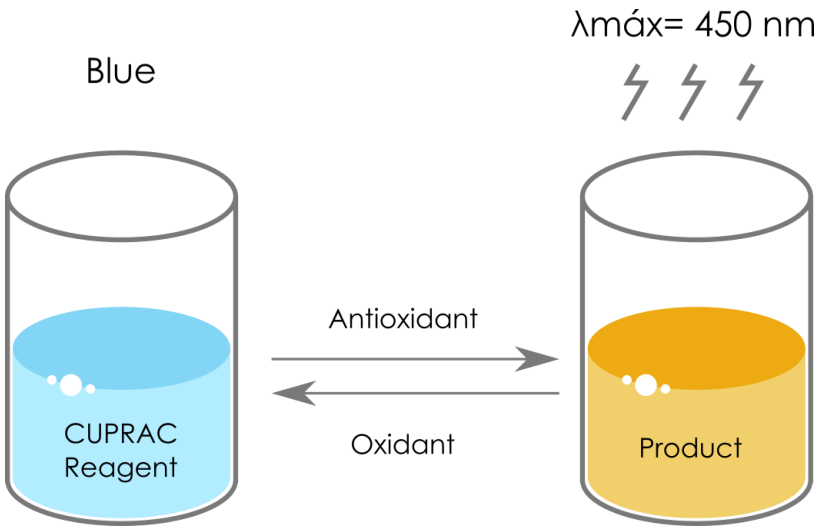
Free radicals are highly reactive species that are naturally formed during cell metabolism in the mitochondria, secretion by immune cells during inflammatory processes or taken up as environmental pollutants.

CUPRAC (CUPric Reducing Antioxidant Capacity) is a mostly new method which enables the Total Antioxidant Capacity (TAC) measurements hydrophilic as well as hydrophobic samples. The main reagent, the copper (II)-neocuproine (2,9-dimethyl-1,10-phenanthroline), is able to oxidize antioxidants generating a coloured product. The chelation with neocuproine enables a fastened reaction by elevating the redox potential of the reagent.

The BQC CUPRAC Antioxidant Capacity Assay Kit is a stable, selective, easy and quick assay that measures all of the significant antioxidants on biological samples at physiological pH.

5. ASSAY PRINCIPLE

On the CUPRAC assay, a redox reduction between the CUPRAC reagent and the antioxidants with a leading thiol group (like for example glutathione) present in the sample, takes place. In this process, the reagent reduces itself forming a chelate complex of copper (I) – neocuproine, which provides a colour measurable at 450 nm in a spectrophotometer.



6. SAMPLE PREPARATION

BQCkit have tested the samples indicated below.

Sample	Preparation required	Dilution factor	Diluent	Long term storage
Wine	No	100	ddH_2O	-20 ºC
Plasma	Yes	-	-	-20 ºC

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 9.

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.

Dilute your sample to an absorbance value corresponding to 0.75-1.5 mM of standard approximately. Filter your sample if it has solid particles in suspension as it might be the case for tea and juices. Store the samples frozen until the day of the assay and avoid contact with air, light and heat. Avoid the formation of bubbles and high speed with the vortex mixer to minimize the oxygen exposure.Plasma:

Mix 60 µl of your plasma sample to 120 µl of Reagent D. centrifuge at 5000 rpm for 10 min. collect supernatant to assay or freeze until the day of the assay.

Dilution of plasma is usually not required. This protocol should be repeated for every replicate with a different microtube for each.

SAMPLE BLANK

It is recommended to run a sample blank when the sample shows absorbance at 450 nm. Follow the Assay Protocol for the sample blank as indicated.

7. ASSAY PREPARATION

REAGENT PREPARATION

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

- Solution B: Add exactly 5 mL of Reagent E to the vials of Reagent B that are going to be used immediately and mix well. Discard the remaining solution.

STANDARD PREPARATION

Add exactly 1 ml of Reagent E 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 E (µl)	TEAC (mM)
1	0	500	0
2	12.5	487.5	0.25
3	25	475	0.5
4	50	450	1
5	75	425	1.5
6	100	400	2

Antioxidant activity is expressed as TEAC (Trolox Equivalent Antioxidant Capacity).

PLATE SET UP

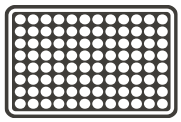
This scheme is just a recommendation on how to perform the assay. For optimal results, BQCkit recommends running the standards and the samples at least for duplicate, but it is the user´s discretion to do so.

	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.

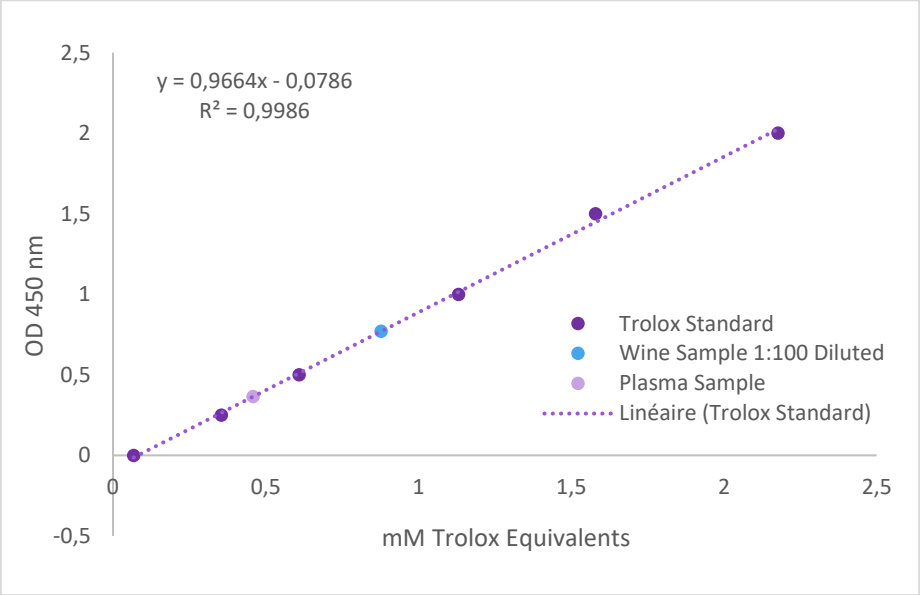
1		Set up the plate design, you can use the BQCkit recommended set up (refer to section Plate set up on page 12) or use your own (refer to section Researcher notes on page 18)
2		Add 40 µl of the sample or standard previously prepared (refer to sections Sample preparation on page 9 and Standard preparation on page 11)
3		Samples and standard: Add 40 µl of Reagent A. Sample Blank: Add 40 µl of Reagent C
4		Samples and standard: Add 40 µl of Solution B previously prepared (refer to section Reagent preparation on page 11). Now your standard wells should become yellow-orange. Sample Blank: Add 40 µl of Reagent C
5		Add 40 µl of Reagent C to every well
6		Add 40 µl of ddH_2O to every well
7		Let the reaction run for 30 minutes.
8		Read the absorbance at 450 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 A 450 nm (y-axis) vs. standard, mM Trolox (x-axis).



ANALYSIS OF THE SAMPLE

Determine the unknown sample concentration using the standard curve from the assayed sample value. If a sample blank was performed, subtract the value for Average the OD for the replicates and then apply:

mM Trolox Equivalents =

(OD 450 nm-intercept/slope)*dilution factor

10. INTERFERING SUBSTANCES

To the best of our knowledge, no interfering substances have been founded.

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												
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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

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