ORAC ASSAY KIT

KF01004

96/192/960 TESTS

96 well plate

BQCkit

A brand of BioQuoChem

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:

96 tests: 12 standard, 42 samples

192 tests: 12 standard, 90 samples

960 tests: 12 standard, 474 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:

25 µl/test

Types of sample compatible:

Biological fluids, cell lysates, tissue homogenates, foods and beverages.

Linear range:

10 – 100 uM

Type of detection:

Fluorimetric (Ex: 485 nm/Em: 528-538 nm)

Sensitivity:

0.20 AUC/uM trolox

Time required for the assay:

150 min

3. MATERIALS

MATERIALS SUPPLIED

Store kit components as indicated below:

96 tests

Product	Nº bottles	Amount	Storage (before use)	Storage (after use)
Reagent A	1	25 ml	-20°C	4°C
Reagent B	1	0.250 ml	-20°C	-
Reagent C	1	Powder	-20°C	-
Reagent D	1	20 µl	-20°C	4°C
Standard	1	Powder	-20°C	-
96-well plate	1	-	-	-

192 tests

Product	Nº bottles	Amount	Storage (before use)	Storage (after use)
Reagent A	1	50 ml	-20°C	4°C
Reagent B	1	0.500 ml	-20°C	-
Reagent C	2	Powder	-20°C	-
Reagent D	1	40 µl	-20°C	4°C
Standard	2	Powder	-20°C	-
96-well plate	1	-	-	-

960 tests

Product	Nº bottles	Amount	Storage (before use)	Storage (after use)

Reagent A	2	125 ml	-20°C	4°C
Reagent B	1	2.5 ml	-20°C	-
Reagent C	5	Powder	-20°C	-
Reagent D	1	100 µl	-20°C	4°C
Standard	5	Powder	-20°C	-
96-well plate	1	-	-	-

MATERIALS NEEDED BUT NOT SUPPLIED

Materials:

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

Instrumentation:

- Microcentrifuge
- Vortex mixer
- Fluorimetric microplate reader – equipped with filter for Ex: 485 nm/Em: 528-538 nm.

4. INTRODUCTION

Antioxidant capacity is an overall ability of organisms or food to catch free radicals and prevent their harmful effect. Antioxidative effect includes protection of cells and cellular structures against harmful effect of free radicals, especially oxygen and nitrogen. Substances with antioxidative properties are called antioxidants. They are contained in food and food supplements, most commonly in fruits, vegetables, rice, wine, meat, eggs, and other foodstuff of plant and animal origin.

Antioxidative systems include antioxidative enzymes, that is, superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, and nonenzymatic substrates, such as glutathione, uric acid, lipoic acid, bilirubin, coenzyme Q, vitamin C (L-ascorbic acid), vitamin A (retinol), vitamin E (tocopherol), flavonoids, carotenoids, teine compounds in green tea, and others. Some biomolecules are also considered biologically active and clinically significant antioxidants, for example, transferrin, ferritin, lactoferrin, ceruloplasmin, hemopexin, haptoglobin, and uric acid.

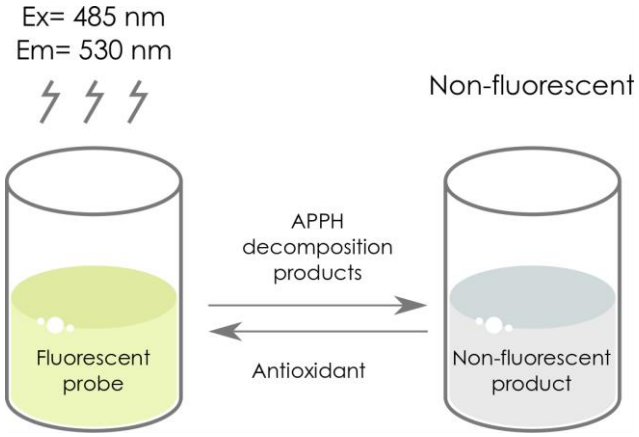
BQC ORAC Kit is a reproducible and simple assay to determine antioxidant capacity on various sample types.

5. ASSAY PRINCIPLE

The ORAC assay depends on the free radical damage to a fluorescent probe, to result in a change of fluorescent intensity and the degree of change is indicative of the amount of radical damage. The presence of antioxidants results in an inhibition in the free radical damage of the fluorescent compound. This inhibition is observed as a preservation of the fluorescent signal.

It is possible quantitate the protection by calculating the area under the curve (AUC) from the experimental sample. After subtracting the AUC for the blank, the resultant difference would be the protection conferred by the antioxidant compound. Trolox®, a water-soluble vitamin E analog, is used as the calibration standard and ORAC results are expressed as Trolox® equivalents.

The ORAC assay is unique because the assay is driven to completion the AUC calculation combines both the inhibition time as well inhibition percentage of free radical damage by the antioxidant into a single quantity.



6. SAMPLE PREPARATION

BQCkit have tested the samples indicated below.

Sample	Preparation required	Dilution factor	Diluent	Long term storage
Cells	Yes			-80 °C
Tissue	Yes			-80 °C
Plasma, serum, saliva	No			-80 °C
Food	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.

Tissue homogenate:

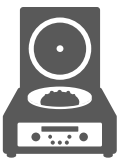
1

Rinse tissue with PBS (pH 7.4)

2

Homogenize 200 mg in 1 ml of cold Reagent A

3



Centrifuge at 5,000 xg for 15 min at 4 °C

4

Collect supernatant to assay or freeze

Total time required: 30 min

Cell lysate:

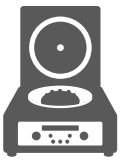
1

Obtain a cell pellet (1 x106 cells). If cells are adherent, please use scraper technique to obtain it.

2

Mix cells with 1 mL of cold Reagent A. To lysis use homogenization or sonication on ice.

3



Then, centrifuge between 1 and 5,000 x g to prepare a cell pellet.

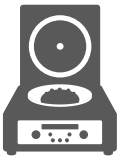
4

Collect supernatant to assay or freeze

Total time required: 35 min

Plasma:

1



2

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

Collect the supernatant to assay or freeze

Total time required: 20 min

Additional notes:

Food:

1

Homogenize solid food in a small volume of cold Reagent A. Store at -80°C until it is used.

Total time required: 10 min

2

Store at -80°C until it is used.

7. ASSAY PREPARATION

REAGENT PREPARATION

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

- **Solution B:** In a separate tube, mix 250 µl of Reagent B with 15 ml of Reagent A. This solution is enough for 96 tests and remains stable in the fridge for few hours.
- **Solution C:** Add 3 ml of Reagent A to the vial of Reagent C and mix. This reagent is stable only for a few hours, discard remaining solution after use.

STANDARD PREPARATION

Add exactly 20 µl of Reagent D to the standard vial. Mix well and add 980 µl of Reagent A. Then, dilute this solution 1:100 with Reagent A. For example: 10 µl standard + 990 µl Reagent A.

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

	Standard (µl)	Reagent A (µl)	Concentration (µM)
1	0	100	0
2	10	90	10
3	25	75	25
4	50	50	50
5	75	25	75
6	100	0	100

Antioxidant activity is expressed as µM Trolox equivalent (TEAC). These values are related to Trolox standard concentration.

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.

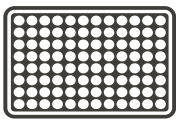
We recommend surrounding the wells that are going to be used with 300 µl of water so evaporation does not affect results.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		S1	S1	C1	C1	C7	C7	C13	C13	C19	C19	
C		S2	S2	C2	C2	C8	C8	C14	C14	C20	C20	
D		S3	S3	C3	C3	C9	C9	C15	C15	C21	C21	
E		S4	S4	C4	C4	C10	C10	C16	C16	C22	C22	
F		S5	S5	C5	C5	C11	C11	C17	C17	C23	C23	
G		S6	S6	C6	C6	C12	C12	C18	C18	C24	C24	
H												

S1-S6: Standard; C1-C24: Samples

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 **Antioxidant activity is** expressed as µM Trolox equivalent (TEAC). These values are related to Trolox standard concentration.

Plate set up on page 13) or use your own (refer to section **Researcher notes** on page 25)

2 

Equilibrate the plate reader incubation chamber to 37°C before beginning. Set-up plate reader to perform a kinetic read for 90 minutes with 1 minute intervals. Excitation = 485 nm; Emission = 528 - 538 nm

3 

Add 25 µl of the sample or standard previously prepared (refer to sections **Sample preparation** on page 10 and **Standard preparation** on page 13)

4 

Add 150 µl of Solution B previously prepared (refer to section **Reagent**

preparation on page 13) in each sample and standard well.

5  Incubate at 37 °C for 30 minutes

6  Add 25 µl of Solution C previously prepared (refer to section **Reagent preparation** on page 13) in each sample and standard well.

7  Read the fluorescence for 90 minutes

9. DATA ANALYSIS

Calculate normalized values for each well at each point time as the fluorescence value divided by the first measurement:

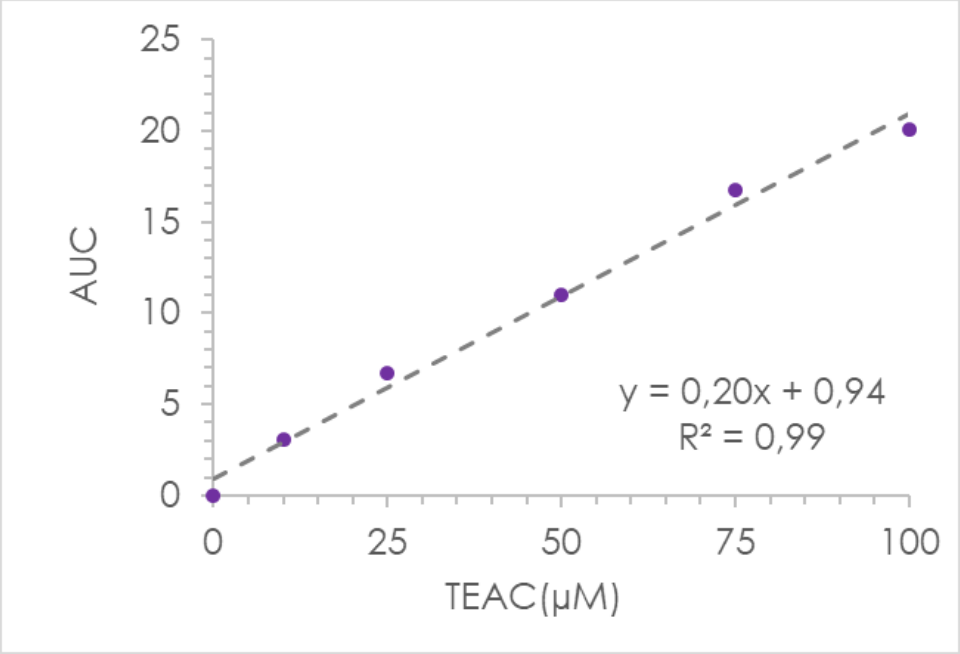
$$RFn = Ft=n/Ft=0.$$

Calculate the area under the curve for each well with the following formula:

$$AUC=0.5+RF1+RF2+RF3...RF88+RF89+(RF90/2)$$

ANALYSIS OF THE STANDARD

Calculate the mean of each point of the standard (experiment recommended to be performed in duplicates). Subtract blank value S1 from the rest of the values of the standard. Then, create a standard curve by plotting the AUC (y-axis) vs. standard, µM Trolox (x-axis).



ANALYSIS OF THE SAMPLE

Calculate the mean of each point of the sample (experiment recommended to be performed in duplicates). Subtract blank value S1 from the rest of the values of the samples.

Determine the unknown sample concentration using the standard curve from the assayed sample value.

$$\text{Value} = \left(\frac{\text{AUC-intercept}}{\text{slope}}\right) * \text{dilution factor}$$

10. INTERFERING SUBSTANCES

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

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
KF01001	DMPD Antioxidant Capacity Assay Kit
KF01002	ABTS Antioxidant Capacity Assay Kit
KF01003	FRAP Antioxidant Capacity Assay Kit
KF01005	CUPRAC Antioxidant Capacity Assay Kit
KF01006	Fast FRAP Antioxidant Capacity Assay Kit
KF01007	DPPH Antioxidant Capacity 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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15. WARRANTIES AND LIMITATION OF LIABILITY

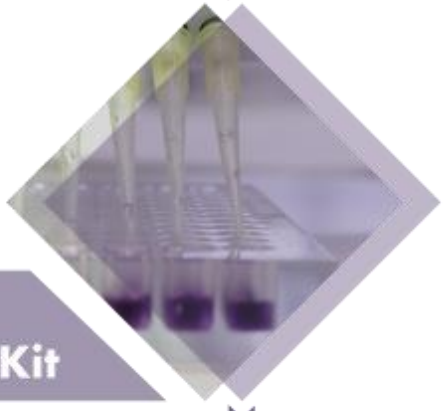
Bioquochem shall not in any event be liable for incidental, consequential or special damages of any kind resulting from any use or failure of the products, even if Bioquochem has been advised of the possibility of such damage including, without limitation, liability for loss of use, loss of work in progress,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