ORAC Assay Kit

KF-01-004

96/192/960 test (96 well plate)

BQCkit

A brand of Bio QuChem

Index

All chemicals should be handled with care

- This kit is for R&D use only

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.

Materials

BQCkit ORAC Assay kit *KF01004-96* contains:

Product	Quantity	Storage
ORAC Reagent A	1 bottle	RT
ORAC Reagent B	1 bottle	4ºC
ORAC Reagent C*	1 bottle	-20ºC
ORAC Standard*	1 vial	-20ºC

BQCkit ORAC Assay kit *KF01004-192* contains:

Product	Quantity	Storage
ORAC Reagent A	1 bottle	RT
ORAC Reagent B	1 bottle	4ºC
ORAC Reagent C*	2 bottles	-20ºC
ORAC Standard*	2 vials	-20ºC

BQCkit ORAC Assay kit *KF01004-960* contains:

Product	Quantity	Storage
ORAC Reagent A	2 bottles	RT
ORAC Reagent B	2 bottles	4ºC
ORAC Reagent C*	2 bottless	-20ºC
ORAC Standard*	4 vials	-20ºC

*These reagents are stable during 10 days at 4ºC and are shipped in these conditions. Once received, is recommended to keep them at -20ºC.

Assay Principle

The ORAC assay depends on the free radical damage to a fluorescent probe, such as fluorescein, 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 to 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®, (6-hydroxy-2,5,7,8-tetrametmethylchroman-2-carboxylic acid) 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 in that 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.

Assay Protocol

Reagents Preparation

96 assay format

Solution 1:

In a 15 mL tube (not included), mix exactly 12.5 mL of Reagent A with 125 µL of Reagent B and mix thoroughly. Once prepared, keep it refrigerated at -20°C.

Solution 2:

Prepare Solution 2 by adding 10 mL of Reagent A in the Reagent C vial. Prepare the Solution 2 immediately prior to performing the assay.

192 assay format

Solution 1:

In a 30 mL tube (not included) mix exactly 25 mL of Reagent A with 250 µL of Reagent B and mix thoroughly. Once prepared, keep it refrigerated at -20°C.

Solution 2:

Prepare solution 2 by adding 10 mL of Reagent A in each Reagent C vial. Prepare the Solution 2 immediately prior to the assay performed.

Assay Protocol

960 assay format

Solution 1:

In 100 mL tubes (not included) mix exactly 62,5 mL of Reagent A with 625 µL of Reagent B and mix thoroughly. Once prepared, keep it refrigerated at -20°C.

Solution 2:

Prepare solution 2 by adding 50 mL of Reagent A in each Reagent C vial. Prepare the Solution 2 immediately prior to the assay performed.

Assay Protocol

Standard Preparation

Antioxidant activity is expressed as µM Trolox® equivalent. These values are related to Trolox® standard concentration. Dilute the Standard Vial 1:10 with water and prepare calibration curve in 1 mL tubes as shown below in Table 1.

Table1. *Reagent volumes needed to carry out the standard curve.*

Standard [µL]	Diluent [µL]	Standard [µM]
0	200	0
20	180	10
40	160	20
60	140	30
80	120	40
100	100	50
120	80	60
140	60	70
160	40	80

Assay Protocol

Sample Preparation

- Cell: Obtain a cell pellet (1 x10^6 cells). If cells are adherent, please use scraper technique to obtain it. Mix cells with 1 mL of cold Reagent A. To lysis use homogenization or sonication on ice. Then, centrifuge between 1 and 5,000 *x g* to prepare a cell pellet. Remove the supernatant and keep on ice to process immediately or store at -80°C until it is used.
- Tissue: Obtain a homogenize tissue samples mixing on an ice bath, 200 mg of tissue with 1 mL of cold Reagent A. Centrifuge at 5,000 *x g* for 15 minutes at 4°C. If it is necessary, centrifuge another time. Remove the supernatant and keep on ice to process immediately or store at -80°C until it is used.
- Plasma, serum, saliva: Store at -80°C until sample is used. It is not necessary any preparation.
- Food: Homogenize solid food in a small volume of cold Reagent A. Store at -80°C until it is used.

Performing the assay

1. 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

2. Dilute your sample to a value corresponding to 10-80 µM of standard approximately.

3. Add 20 µL of the sample or standard in each well.

4. Add 120 µL of Solution 1 previously prepared (see Reagents Preparation) in each well. Place the plate at 37°C during 10 minutes.

5. Add 60 µL of Solution 2 previously prepared (see Reagents Preparation) in each well.

6. Place the plate in the reader and begin kinetic fluorescence reading.

7. Use Bioquochem Excel Worksheet to obtain your results as Trolox equivalents.

Data Analysis

1. Use Area Under the Curve (AUC) obtained with normalized values to calculate standard curve. AUC values can be calculated by the following formula: AUC= 0.5 + (F1/F0) + (F2/F0) + …+ 0.5*(F30/F0) Where F0= normalized fluorescence at t=0.

2. Calculate the ORAC value of the samples (µM Trolox® equivalents) using the equation obtained from the linear regression of the standard curve substituted AUC values for each sample.

Trolox® equivalents (µM) = (AUC– intercept)/ slope

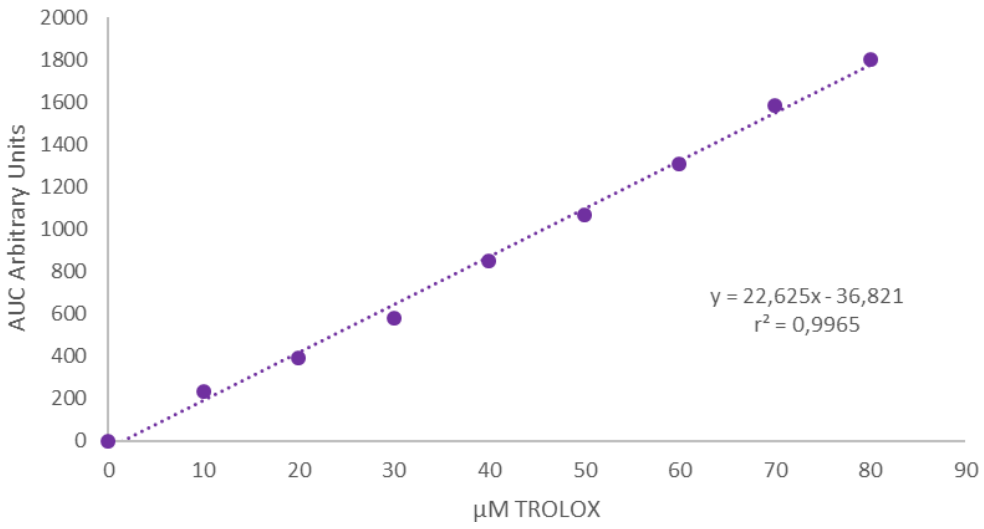

Figure 1. *Example of the standard representation*

3. If you use Bioquochem Excel Worksheet provided with this kit, all these calculations are automatized.

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.