# KF01004
# ORAC Antioxidant Capacity Assay Kit

**96 well plate**
**100/200/400 tests**

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# 1. General information

## PRECAUTIONS

Please read this manual carefully before beginning the assay.

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. It is recommended to use basic Personal Protective Equipment.

Do not use after the expiration date stated on the packaging.

Do not mix or substitute reagents or materials from other kit batches or vendors.

For the **material safety data sheet** (MSDS) please contact us at info@bioquochem.com

## TECHNICAL RECOMMENDATIONS

Store reagents as indicated in **Materials and storage** section.

Be sure to keep the bottle capped when not in use.

Let the components reach room temperature (RT) before use.

Immediately before use, gently invert and rotate reagent bottles several times to mix the contents thoroughly.

Avoid foaming or bubbles when mixing or reconstituting components.

Avoid cross contamination of samples or reagents by changing pipette tips between sample, standard and reagent additions.

Be sure to use the optimal microplate for the assay. Flat bottom transparent microplates for UV/VIS applications, and black microplates for fluorescence measurements.

# 2. Technical specifications

### Available sizes

100/200/400 tests

### Required sample volume

20 µL/test

### Compatible samples

Biological fluids, tissue homogenates, cell lysates, food, and beverages

### Type of detection

Fluorimetric (Ex.: 485 nm/Em.: 528 nm)

# 3. Materials and storage

## MATERIALS SUPPLIED

| Item | No. Tests | Units | Storage |
|---|---|---|---|
| Reagent A | 100 | 1 | 4 °C |
| | 200 | 1 | |
| | 400 | 2 | |
| Reagent B | 100 | 1 | -20 °C |
| | 200 | 2 | |
| | 400 | 4 | |
| Reagent C | 100 | 1 | 4 °C |
| | 200 | 2 | |
| | 400 | 4 | |
| Standard | 100 | 1 | -20 °C |
| | 200 | 2 | |
| | 400 | 4 | |
| Black 96-Well Microplate | 100 | 1 | RT |
| | 200 | 2 | |
| | 400 | 4 | |

## MATERIALS NEEDED BUT NOT SUPPLIED

- Double distilled water (dd$H_2O$) as Milli-Q Ultrapure Water.
- Labware materials (micropipettes, tubes, stirring/mixing equipment).
- Fluorimetric microplate reader – equipped with filter for Ex.: 485 nm/Em.: 528 nm.

## STORAGE CONDITIONS

On receipt, store kit components as indicated above. Under these conditions, the reagents are stable in the original packaging until the expiration date indicated on the outside of the box. After reconstitution, standard solutions are unstable in the presence of oxygen. Prepare a fresh set of standards for every use.

# 4. Introduction

Antioxidants serve as a protection against the harmful effects of free radical damage. Antioxidant systems include both antioxidative enzymes (superoxide dismutase, catalase, glutathione peroxidase, etc.), and low-molecular weight non-enzymatic compounds (glutathione, uric acid, lipoic acid, bilirubin, coenzyme Q, vitamin C, vitamin A, vitamin E, flavonoids, carotenoids, etc.).

Total antioxidant capacity (TAC) is a global measure of the non-enzymatic antioxidant efficiency that integrates the individual effect of all antioxidants in a given matrix, and their additive, synergistic or antagonistic interactions.

TAC is considered as an important parameter to establish antioxidant status of biological samples. Alterations in the redox status of tissue/organs and body fluids have been linked to several health impairments such as infertility, obesity, cancer, and neurodegenerative diseases.

TAC is also an important tool for plant characterization and for food quality control since the antioxidant levels vary depending on environmental factors, harvesting, aging, storage conditions, etc.

**BQC ORAC (Oxygen Radical Antioxidant Capacity) Assay Kit is a powerful tool to measure the antioxidant capacity of biological samples, cell lysates, tissue homogenates, food, and beverages.**

# 5. Assay principle

The ORAC Assay is based on the inhibition of fluorescein oxidation by peroxyl radical in the presence of antioxidants. Peroxyl radical (ROO·) is produced by a free radical initiator (AAPH: 2,2'-azobis-2- methyl-propanimidamide dihydrochloride) which produces a fluorescent quenching of fluorescein over time. The presence of antioxidants inhibits the free radical damage to fluorescein, resulting in a preservation of the fluorescent signal.

*Principle of ORAC Assay Kit*

The sample antioxidant capacity correlates to the area under the fluorescence decay curve (AUC). The AUC is used to determine the antioxidant activity in a sample and is compared to an antioxidant standard curve prepared using the water-soluble vitamin E analog Trolox as standard. ORAC results are expressed as Trolox equivalents (TEAC).

# 6. Assay preparation

## REAGENT PREPARATION

**All assay reagents not listed below are ready to use as supplied. Allow the reagents to reach room temperature before use.**

**R.B. Working Solution:** Preparation of this Working Solution requires **two dilution steps.**

**1)** Dilute Reagent B 1:100 with Reagent A (e.g. 10 µL of Reagent B with 990 µL of Reagent A) and mix well.

**2)** Dilute the 1:100 diluted Reagent B solution 1:40 with Reagent A to prepare the R.B. Working Solution (e.g. for 100 tests, prepare 16 mL of R.B. Working Solution by diluting 0.4 mL of 1:100 diluted Reagent B with 15.6 mL of Reagent A.

**CAUTION:** R.B. Working Solution must be prepared immediately before use. This solution remains stable at 4 °C for a few hours. Keep the solution protected from the light.

**R.C. Working Solution:** Add 8 mL of Reagent A to the vial of Reagent C and mix thoroughly.

**CAUTION:** R.C. Working Solution must be prepared immediately before use. This solution is stable only for a few hours.

**Standard Solution (Trolox):** Add 1 mL of Reagent A to the Standard vial. Mix well. Dilute this standard solution 1:40 with Reagent A (e.g. 25 µL of standard solution with 975 µL of Reagent A). Use this diluted solution to prepare the standard curve.

## STANDARD CALIBRATION

Prepare Trolox (TX) standards for the calibration curve from the 1:40 diluted Standard solution according to the following Table. Prepare the standards immediately prior to each assay. Vortex tubes thoroughly. Discard standard solutions after use.

| Standard | Standard solution 1:40 diluted (μL) | Reagent A (μL) | *TEAC (μM TX) |
|---|---|---|---|
| Std 1 (Reagent Blank) | 0 | 250 | **0** |
| Std 2 | 10 | 240 | **10** |
| Std 3 | 25 | 225 | **25** |
| Std 4 | 50 | 200 | **50** |
| Std 5 | 100 | 150 | **100** |
| Std 6 | 175 | 75 | **175** |

*Antioxidant activity is expressed as TEAC (Trolox Equivalents Antioxidant Capacity).

## PLATE SET UP

**BQC recommends running the standards and samples at least in duplicate (triplicate recommended).** There is no specific pattern for using the wells on the plate. A proposed layout of standards (Std) and samples (S) to be measured in duplicate is shown below.

**NOTE:** If sample blanks are included in the assay, it is necessary to reserve some wells of the plate for these blanks.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Std 1 | Std 1 | S2 | S2 | S10 | S10 | S18 | S18 | S26 | S26 | S34 | S34 |
| B | Std 2 | Std 2 | S3 | S3 | S11 | S11 | S19 | S19 | S27 | S27 | S35 | S35 |
| C | Std 3 | Std 3 | S4 | S4 | S12 | S12 | S20 | S20 | S28 | S28 | S36 | S36 |
| D | Std 4 | Std 4 | S5 | S5 | S13 | S13 | S21 | S21 | S29 | S29 | S37 | S37 |
| E | Std 5 | Std 5 | S6 | S6 | S14 | S14 | S22 | S22 | S30 | S30 | S38 | S38 |
| F | Std 6 | Std 6 | S7 | S7 | S15 | S15 | S23 | S23 | S31 | S31 | S39 | S39 |
| G | Std 7 | Std 7 | S8 | S8 | S16 | S16 | S24 | S24 | S32 | S32 | S40 | S40 |
| H | S1 | S1 | S9 | S9 | S17 | S17 | S25 | S25 | S33 | S33 | S41 | S41 |

*Example of plate layout for the ORAC Assay Kit*

# 7. Sample preparation

**The following sample preparation protocols are intended as a guide only. The optimal conditions for sample preparation must be determined by the end user.** It is recommended to use fresh samples. If it is not possible, aliquot and store samples appropriately with minimal freeze/thawing.

**ORAC Assay Kit can be used to determine TAC in a wide variety of samples like biological fluids, tissue homogenates, cell lysates, food, and beverages.**

**Biological samples.** Biological samples like heparinized plasma, serum or urine can be directly measured with appropriate dilutions.

**Tissue Homogenates.** Dissect the tissue of interest and place it on a homogenizer tube with an appropriate amount of an ice-cold buffer. Homogenize the tissue and then centrifuge the homogenate at 10000 x g for 15 minutes at 4 °C. Collect the supernatant.

**Cell culture.** Wash cells with ice-cold buffer (e.g. PBS) before lysis. Lyse cells by sonication or freeze-thaw cycles. Centrifuge cell lysis suspension at 10000 x g for 15 minutes at 4 °C and collect the supernatant.

**Food and beverages.** Fruit juices and other beverages such as wine, tea, and coffee can be directly measured with appropriate dilutions. If it is required, filter (0.2 µm pore size) the samples prior performing the assay. For the analysis of other food samples an extraction step is usually required. The extraction method varies based upon the sample type. The most common extraction solvents include acid/methanol, acid/ethanol, or acetone. If it is required clarify the sample through either filtration or centrifugation prior performing the assay.

Reagents and materials required for sample preparation are not supplied with the kit. Before doing sample preparation, consider the volume of sample required per test; see **Technical specifications** section.

Make sure that interfering substances present in the sample do not give a significant background. Run proper blanks as necessary. It is recommended to assay different sample dilutions to ensure the values fall within the standard curve.

# 8. Assay protocol

**Prepare and mix all reagents thoroughly before use. Each standard, sample or blank should be assayed at least in duplicate.**

1. Equilibrate the plate reader incubation chamber to 37 °C before beginning
2. Set up the plate design
3. Add **20 µL** of standard or sample in each well
4. Add **120 µL** of **R.B. Working Solution** in each well and mix
5. **Incubate** the plate for **30 minutes** at **37 °C**
6. Standard and sample wells: Add **60 µL** of **R.C. Working Solution**
7. **Shake the plate slowly for 30 seconds** before starting the measurement(if possible)
8. Read the **fluorescence** (Ex.: 485 nm/Em.: 528 nm) of all wells for **90 minutes** in intervals of 1 minute at **37 °C** (total reads: 91)

   ! Select the right gain for the measurement

If you need to **adapt this kit** for another form of the assay (for example cuvette), **contact us at** info@bioquochem.com

# 9. Data analysis

- Calculate relative fluorescence value at each time point ($RF_n$) as follows: $RF_n = \frac{F_n}{F_1}$

  ***$F_n$*** is the fluorescence value at each time point

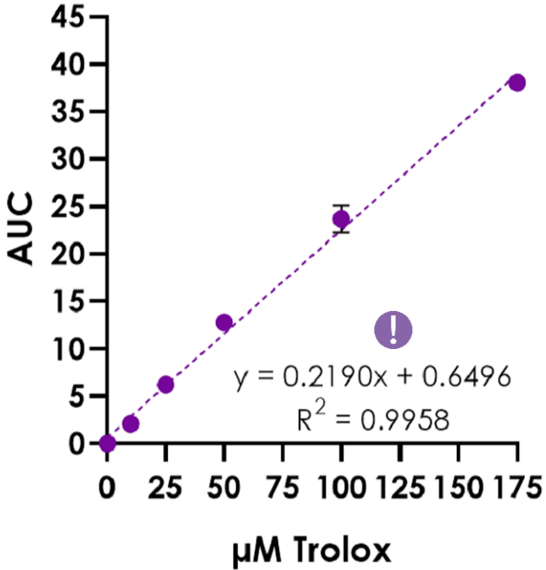
  ***$F_1$*** is the fluorescence value at time 0

- Use this normalized data to calculate the area under the curve (AUC) for each well with the following formula:

$$AUC = 0.5 + \sum_{1}^{90} RF + \frac{RF_{91}}{2}$$

## ANALYSIS OF THE STANDARDS

- Calculate the average AUC of all the standards.
- Subtract the average AUC of the reagent blank (Std 1) from the average AUC of all the standards to obtain the blank-corrected AUC of the standards.
- Create a standard curve by plotting the blank-corrected AUC of the standards as a function of the standard concentration (see **STANDARD CALIBRATION** section). A typical standard curve (**y**=slope·**x** ± intercept) for this assay is shown below.




*TX standard curve with ORAC Assay Kit*

❗ This standard curve is an example of the data typically obtained with this kit. **DO NOT USE** this standard curve to calculate the TAC of your samples. A new standard curve must be performed by the end user.

## ANALYSIS OF THE SAMPLES

- Calculate the average AUC of the samples.
- Subtract the average AUC of the reagent blank (Std 1) from the average AUC of each sample to obtain the blank-corrected AUC of the samples ($AUC_S$).
- Calculate the TAC value of the samples using the following equation. Slope and intercept values are obtained from the standard curve.

$$\text{TEAC } (\mu\text{M TX}) = \left(\frac{AUC_S - \text{intercept}}{\text{slope}}\right)$$

When working with diluted samples the concentration values obtained must be multiplied by the dilution factor to obtain the TEAC (µM TX) value of the undiluted sample.

# 10. Troubleshooting

This troubleshooting table provides potential sources and solutions for common problems observed with BQC Assay Kits. **The problems listed below could occur when using any BQC Assay Kit**. They are not specific for this assay kit.

| Problem | Possible Cause | Recommended Solution |
|---|---|---|
| Wells have color but there is no reading | Plate read at incorrect wavelength | Check the wavelength used in the assay |
| | Incorrect microplate | Use the correct microplate for your application<br>UV/Vis: transparent<br>Fluorescence: black wells/transparent bottom |
| Standard readings do not follow a linear pattern | Pipetting errors in preparation of standards | Avoid pipetting small volumes (<5 µL)<br>Be careful not to splash from well to well |
| | Air bubbles formed in well(s) | Use reverse pipetting technique |
| | Standard stock is at incorrect concentration | Always refer to dilutions described in **Assay preparation** |
| | Improperly thawed reagents | Thaw all components completely and mix well before use |
| | Use of improperly stored reagents | Store the components appropriately<br>Use fresh components from the standard curve |
| | Incorrect incubation times or temperatures | Refer to **Assay protocol** |
| Dispersion of standard and sample readings | Pipetting errors | Avoid pipetting small volumes (<5 µL)<br>Be careful not to splash from well to well |
| | Air bubbles formed in well(s) | Use reverse pipetting technique |

| Problem | Possible Cause | Recommended Solution |
|---|---|---|
| Sample erratic values | Samples contain interfering substances | Dilute sample further (if possible) |
| | Inappropriately stored samples or samples used after multiple freeze-thaw cycles | Use fresh samples or store appropriately until use |
| | Samples not deproteinized | Use an appropriate deproteinization protocol |
| | Cells/Tissue samples not homogenized completely | Repeat the sample homogenization |
| | Inappropriate sample dilution buffer | Refer to **Assay preparation** |
| Sample reading fall outside the detection range | Samples are too diluted/concentrated<br>No analyte/activity is observed in the sample | Re-assay using different sample dilutions |

## STILL HAVING PROBLEMS?

Contact BQC if you have any further questions, our team will be pleased to help you:

| | | |
|---|---|---|
| Phone | + 34 985 26 92 92 |
| E-mail | info@bioquochem.com |
| Business hours | Monday-Thursday: 8.30 to 17.00 (CEST)<br>Friday: 8.00 to 15.00 (CEST) |

# 11. Additional information

**ORAC Assay Kit** is a sensitive and precise assay (RSD< 5 %) for determining TAC in a wide variety of samples.

Metallic ions and plasmatic proteins have been reported to interfere with this assay. If these substances are found in the sample, remove them, use EDTA to attenuate interferences or if it is not possible, dilute sample further.

If unexpected results are obtained running your samples, please contact us at info@bioquochem.com

# 12. Related products

More products available on bioquochem.com

| Reference | Product |
|---|---|
| KB03006 | Polyphenol Quantification Assay Kit |
| KB03027 | PCA Deproteinizing Assay Kit |
| KB03030 | Sulfosalicylic Acid Deproteinizing Assay Kit |

# 13. Warranties and limitation of liability

BQC 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 BQC 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 BQC´s gross negligence. Any and all liability of BQC hereunder shall be limited to the amounts paid by the buyer for the product.

Buyer´s exclusive remedy and BQC´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 BQC within 30 days of shipment.

**Expiration date:** 1 year from the date of fabrication. Expiration date is indicated on the outside of the box.

For further details, please refer to our website **bioquochem.com**

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