# ABTS Assay Kit

*KF-01-002*

*250 tests (96 well plate)*

BQCkit

A brand of Bio QuChem

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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 ABTS Assay Kit *KF-01-002 250 tests (96 well plate)* contains:

| Product | Quantity | Storage |
|---|---|---|
| Reagent A | 5 vials | -20 ºC |
| Reagent B | 1 bottle | RT |
| Standard | 2 vials | RT |

Note 1: Each vial of Reagent A is valid for 50 tests. Discard the remaining solution.
Note 2: Each vial of Standard is valid for 125 tests. Discard the remaining solution.

## *Assay Principle*

Bioquochem ABTS assay kit is recommended for total antioxidant activity of solutions of pure substances, aqueous mixtures and beverages.

The assay described here involves the direct production of the blue/green ABTS•+ chromophore. This has absorption maxima at 734 nm.

The addition of antioxidants to the pre-formed radical cation, reduces it ABTS depending on the antioxidant activity and the concentration of the antioxidant.

In our assay a solution of ABTS at neutral pH and in the presence of a suitable solution, can form a stable and colored radical cation (ABTS•+) which shows a maximum of absorbance at 734 nm. Antioxidant compounds quench the color and produce a decoloration of the solution which is proportional to their amount. This reaction is rapid and the end, which is stable, is taken as a measure of the antioxidative efficiency.

ABTS (uncolored) + Reagent → ABTS• + (blue/green)

(λmáx= 734 nm) ABTS• + (blue/green) + AOH → ABTS (uncolored) + AO

Scheme 1. *Formation of radical ABTS and its reaction with antioxidants (AOH)*

## *Reagent Preparation*

Allow the reagents to reach room temperature.

ABTS Solution:

In a 10 ml tube (not provided), mix 1 vial of Reagent A (1mL) with 9 mL of Reagent B. This solution must have an absorbance of around 0.70 (±0.02) at 734 nm. This solution is called ABTS Solution. Use this solution immediately.

Standard solutions:

For standard solution preparation, add exactly 1 mL of deionized water to each Standard vial. This solution must be freshly prepared.

Dilute 1:10 the Standard Solution previously prepared.

Samples:

Dilute your sample in EtOH (for phenolic compounds and food extracts) or ddH2O (for plasma) such that, after introduction of 5 µL of each aliquot into 200 µL of ABTS Solution, it produces between 5%-35% inhibition of the blank absorbance (ABTS• + alone).

# *Assay Protocol*

## Standard Calibration Curve

Prepare calibration curves in 1.5 mL tubes as shown in Table 1.

NOTE: Keep these tubes in ice during the assay.

Table 1. *Reagent volumes needed to carry out the standard curve*

| | $ddH_2O$ [µL] | Standard [µL] | CEAC* [µM] |
|---|---|---|---|
| S1 (Blank) | 100 | 0 | 0 |
| S2 | 90 | 10 | 100 |
| S3 | 85 | 15 | 150 |
| S4 | 75 | 25 | 250 |
| S5 | 70 | 30 | 300 |
| S6 | 60 | 40 | 400 |
| S7 | 50 | 50 | 500 |
| S8 | 40 | 60 | 600 |

**Antioxidant activity is expressed as CEAC (Vitamin C equivalents antioxidant capacity).*

# *Assay Protocol*

## Plate set up

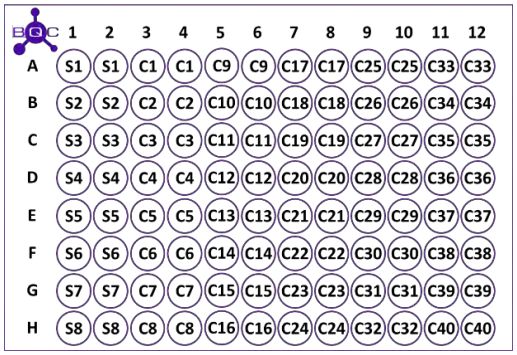



Figure 1. *96-well plate filling format*

S1-S8: Standards

C1-C40 = Samples

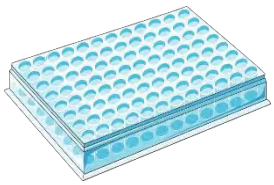
## Attention

- This scheme is just a recommendation of how to perform the assay.
- If the antioxidant activity in the samples is not known or if it is expected to be beyond the range of the standard curve, it is recommended to assay the samples at several dilutions.
- For optimal results, it is recommended to run the standards and the samples for duplicate, but it is the user´s discretion to do so.

The blank sample absorbance ($A_0$) must be ≥ 0.7

# *Assay Protocol*

## Short protocol:

1  Prepare all reagents and 96 well plate.

2  Add 5 µL of the sample standard in each well.

3  Add 200 µL of ABTS Solution you have previously prepared (see Reagent preparation) in each well.

4  Mix the mixture for 5 minutes under continuous stirring.

5  Read the absorbance at 734 nm at about 27ºC.

## *Data Analysis*

1. Calculate the absorbance at 734 nm as percentage of the absorbance of the uninhibited radical cation solution (Blank) according to the equation:

$$\text{Inhibition of } A_{734nm}\ (\%) = (1-(A_f/A_0)) \times 100$$

Where $A_0$ is the absorbance of uninhibited radical cation and $A_f$ is, the absorbance measured 5 min after the addition of antioxidant samples.

2. Plot the inhibition of $A_{734nm}$ of standards as function of their final concentrations (Table 1). See Figure 2 for a typical standard curve.

3. Calculate the CEAC value of the samples using the equation obtained from the linear regression of the standard curve substituted of $A_{734nm}$ values for each sample.

CEAC (µM) = (sample inhibition $A_{734nm}$ - intercept) / slope

# *Data Analysis*

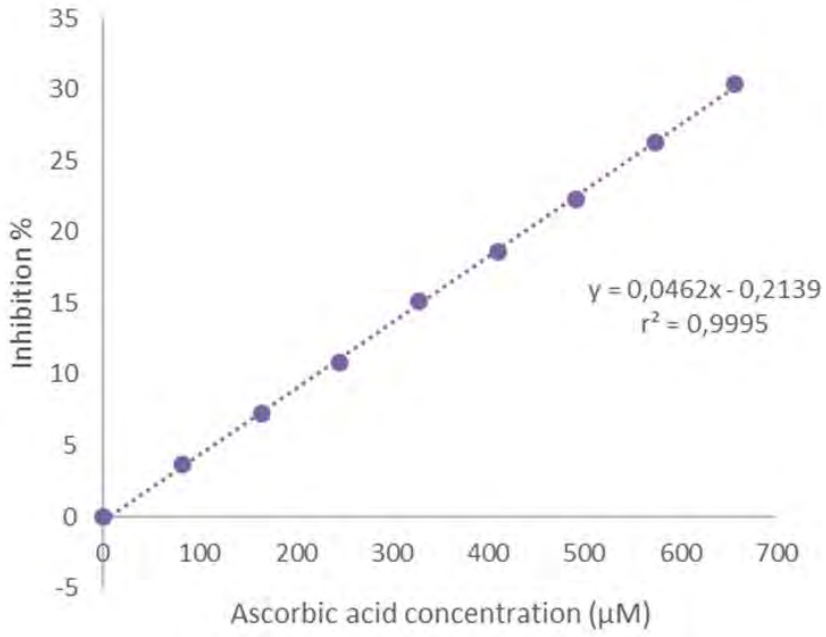



Figure 1. *Typical standard curve for ABTS assay*

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Expiration date: 2 months from the date of delivery

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