DMPD Assay Kit

KF-01-001

200 test (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 DMPD Assay kit *KF01001-200 tests* contains:

Product	Quantity	Storage
DMPD Reagent A	1 vial (powder)	4ºC
DMPD Reagent B*	1 vial	-20ºC
DMPD Reagent C	2 vials	RT
DMPD Reagent D	2 bottles	RT
DMPD Standard*	1 vial (powder)	-20ºC

*These reagents are stable several weeks at 4 degrees and is shipped in these conditions. Once received is recommended to keep them at the temperature shown in the table.

Assay Principle

Bioquochem DMPD assay kit is recommended for antioxidant measurements of beverages. This kit measures the antioxidant activity of compounds that are able to transfer hydrogen atoms.

When the compound N,N-dimethyl-ρ-phenylenediamine (DMPD) is in the presence of a suitable oxidant solution, a colored radical cation is formed (DMPD•+). Antioxidant compounds, which are able to transfer a hydrogen atom to DMPD•+, cause a decoloration of the solution.

In our assay a solution of DMPD at an acidic pH and in the presence of a suitable oxidant solution, can form a stable and colored radical cation (DMPD•+) which shows a maximum of absorbance at 553 nm. Antioxidant compounds which are able to transfer a hydrogen atom to DMPD•+ quench the color and produce a decoloration of the solution which is proportional to their amount. This reaction is rapid and the end point, which is stable, is taken as a measure of the antioxidative efficiency.

$$\text{DMPD (uncolored)} + \text{Oxidant} + H^+ \rightarrow \text{DMPD}^{\bullet} + \text{(purple)}(\lambda_{máx} = 553\ \text{nm})$$
$$\text{DMPD}^{\bullet} + \text{(purple)} + \text{AOH} \rightarrow \text{DMPD(uncolored)} + \text{AO}$$

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

Sample Preparation

If your sample is colored, dilute it to an absorbance value lower than the measure A_0.

Reagent Preparation

Allow the reagents to reach room temperature before use.

Reagent A

Add exactly 1mL of ddH2O in the Reagent A Vial and mix thoroughly. This solution should be kept at -20°C over 1 month.

Standard solution

Add exactly 1 mL of Reagent C in the standard vial. This solution should be kept at -20ºC over 1 month. Then dilute the standard solution 10 times in a 1.5 mL vial (not provided) to perform the calibration curve. This solution should be freshly prepared.

DMPD•+ solution

To each Reagent D bottle, add exactly 300 µL of Reagent A and 60 µL of Reagent B. Mix well and let stand at room temperature for 10 minutes to allow the radical to perform. This solution should be freshly prepared.

Assay Protocol

Standard curve preparation:

Antioxidant activity is expressed as TEAC (Trolox equivalent antioxidant capacity). For this purpose, use the 1:10 diluted standard previously prepared (see Reagents preparation).

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

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

Sample	Reagent C [µL]	Diluted Standard [µL]	TEAC [µg/mL]
S1 (Blank)	500	0	0
S2	485	15	25
S3	465	35	50
S4	433	67	100
S5	416	84	125
S6	400	100	150
S7	366	134	200
S8	333	167	250

Assay Protocol

Plate set up

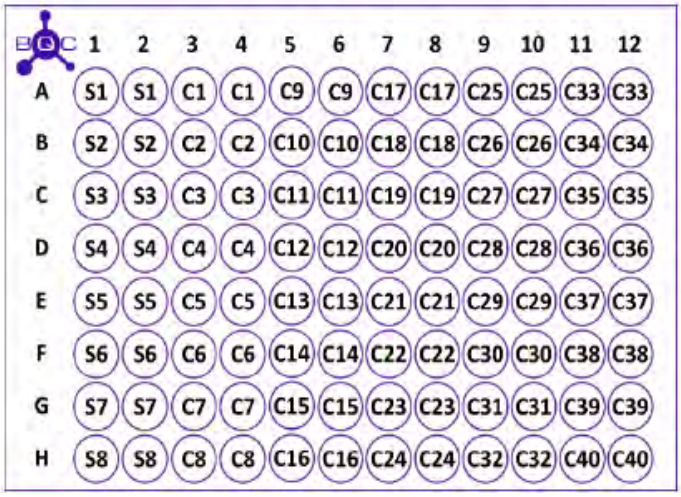

Figure 1. *96-well plate filling format*

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.

Assay Protocol

Performing the assay

1. Add 20 µL of the sample or standard in each well.
2. Add 280 µL of **DMPD•+ solution** (see Reagents preparation) in each well.
3. Mix the mixture at Room Temperature for 10 minutes under continuous stirring.
4. Read the absorbance at 553 nm.

Data Analysis

1. Calculate the absorbance at 553 nm as percentage of the absorbance of the uninhibited radical cation solution (Blank) according to the equation:

$$\text{Inhibition of A553 (\%)} = (1-(Af/A0)) \times 100$$

Where A0 is the absorbance of uninhibited radical cation and Af is, the absorbance measured 10 min after the addition of antioxidant samples.

2. Plot the inhibition of standards as a function of their final concentrations (Table 1). See Figure 2 for a typical standard curve.
3. Calculate the TEAC value of the samples using the equation obtained from the linear regression of the standard curve substituted inhibition percentage values for each sample.

$$\text{TEAC (µg/ ml)} = (\text{sample inhibition A553} - \text{intercept}) / \text{slope}$$

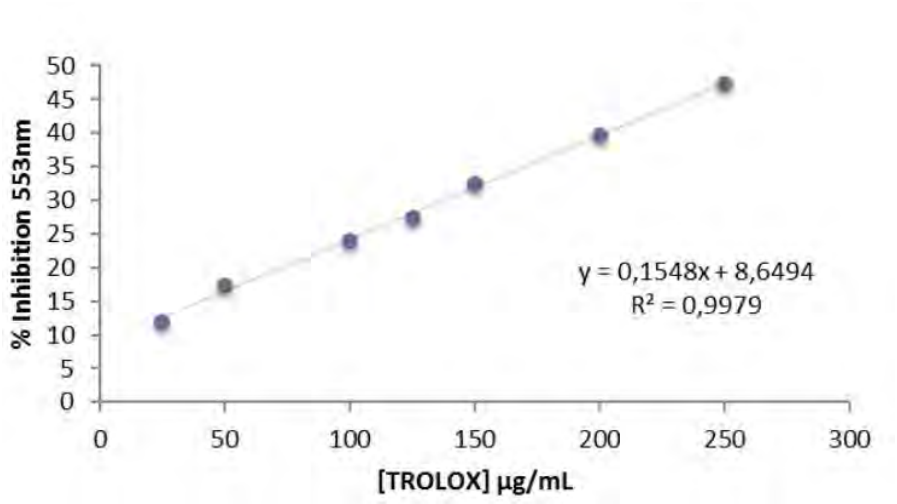

Figure 2. *Typical standard curve for DMPD assay. (This standard curve is just an example, the results of each assays could vary).*

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Expiration date: 1 year from the date of delivery

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