BQC

REDOX TECHNOLOGIES

KF01001
DMPD 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

Beverages, food samples and plant extracts

Type of detection

Colorimetric (553 nm)

3. Materials and storage

MATERIALS SUPPLIED

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

MATERIALS NEEDED BUT NOT SUPPLIED

- Double distilled water (ddH_2O) as Milli-Q Ultrapure Water
- Labware materials (micropipettes, tubes, stirring/mixing equipment)
- Colorimetric microplate reader – equipped with filter for OD 553 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.).

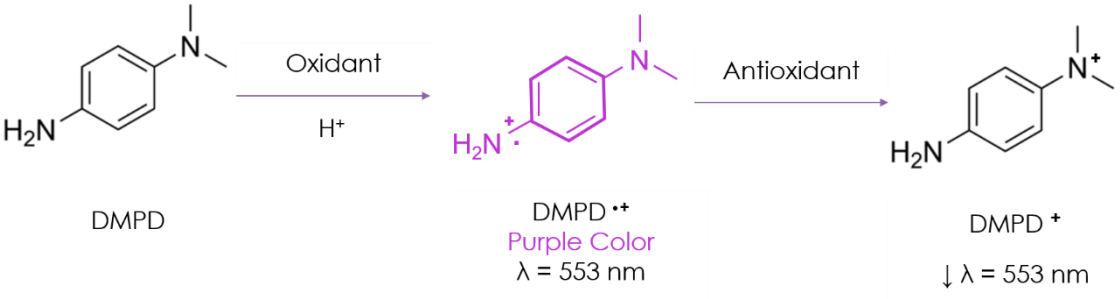
Total antioxidant capacity (TAC) is a global measure of the non-enzymatic antioxidant efficiency that integrates the individual effect of all antioxidants in a given matrix, and their additive, synergistic or antagonistic interactions.

TAC determination is 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 DMPD Antioxidant Capacity Assay Kit is an easy and quick assay to measure TAC in beverages, food samples and plant extracts.

5. Assay principle

This TAC Assay Kit is based on the N,N-dimethyl-p-phenylenediamine (DMPD) method. In this method, the DMPD radical ($DMPD^{\bullet+}$) which is a purple colored (λ_{max} = 533 nm) stable cation radical, is reduced by hydrogen-donating antioxidants to the colorless DMPD reduced form. Therefore, the absorbance decrease at 533 nm depends linearly on the antioxidant concentration. The synthetic antioxidant Trolox (included in the kit) is used to standardize the sample TAC relative to Trolox (Trolox Equivalents Antioxidant Capacity, TEAC).



Principle of DMPD Assay Kit

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.A. Working Solution: Add 500 µL of ddH_2O to the Reagent A vial and mix well.

R.B. Working Solution: Add 500 µL of ddH_2O to the Reagent B vial and mix well.

> **CAUTION:** R.B. Working Solution must be freshly prepared and used immediately

$DMPD^{\bullet+}$ Working Solution: Add 300 µL of R.A. Working Solution and 60 µL of R.B. Working Solution to each Reagent D bottle. Mix well and **let stand for 10 minutes at room temperature.**

> **CAUTION:** $DMPD^{\bullet+}$ Working Solution must be freshly prepared and used immediately

Standard Solution (Trolox): Add 500 µL of Reagent C to the Standard vial and mix well. Dilute this standard solution 1:10 with Reagent C (e.g. 100 µL of standard solution with 900 µL of Reagent C). Use this 1:10 diluted solution to prepare the standard curve.

STANDARD CALIBRATION

Prepare Trolox (TX) standards for the calibration curve from the 1:10 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 1:10 diluted (µL)	Reagent C (µL)	*TEAC (µg TX/mL)
Std 1	0	300	**0**
Std 2	6	294	**10**
Std 3	15	285	**25**
Std 4	30	270	**50**
Std 5	45	255	**75**
Std 6	60	240	**100**

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

PLATE SET UP

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

	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	B	B	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 DMPD 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.

DMPD Assay Kit can be used to determine TAC in beverages, food samples and plant extracts.

Fruit juices and other beverages such as wine, tea, and coffee can be directly measured with appropriate dilutions. If it is required, clarify the sample through filtration prior performing the assay.

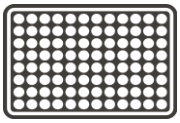
For the analysis of other samples like foods or plant material an extraction step is usually required. The extraction method varies based upon the sample type. The most common extraction solvents include acid/methanol, acid/ethanol, or acetone.

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 (e.g. sample blank should be always evaluated when working with highly colored samples). 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  Set up the plate design

2  Add **20 µL** of **standard** or **sample** in each well.

For **reagent blank** wells add **20 µL** of **Reagent C**

3  **- Standard** and **sample** wells: Add **280 µL** of **DMPD•+ Working Solution**

- Reagent blank wells: Add **280 µL** of **Reagent D**

4  **Incubate** for **10 minutes** at **RT**

5  Read the **absorbance** of all wells at **553 nm** in end point mode at **RT**

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

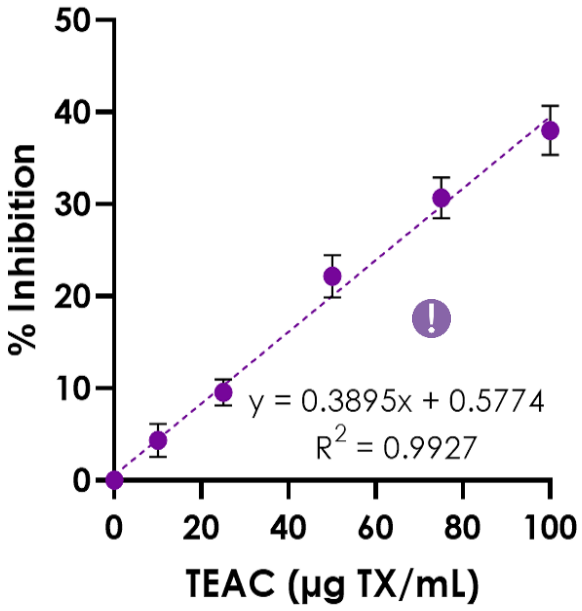
9. Data analysis

ANALYSIS OF THE STANDARDS

- Calculate the average absorbance of all the standards.
- Subtract the average absorbance of the reagent blank from the average absorbance of the standards to obtain the blank-corrected absorbance of the standards (A_{Std}).
- Calculate the % inhibition for the standards according to the following equation:

$$\% \text{ Inhibition of Standard} = \left(1 - \frac{A_{Std}}{A_0}\right) \cdot 100$$

  Where A_0 is the blank-corrected absorbance of Std 1 (0 µg TX/mL) and A_{Std} is the blank-corrected absorbance measured for the remaining standards.
- Create a standard curve by plotting the % inhibition of 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 DMPD 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 absorbance of the samples.
- Subtract the average absorbance of the reagent blank from the average absorbance of each sample to obtain the blank-corrected absorbance of the samples (A_s).
- Calculate the % inhibition for the samples according to the following equation:

$$\% \text{ Inhibition of Sample} = \left(1 - \frac{A_S}{A_0}\right) \cdot 100$$

Where A_0 is the blank-corrected absorbance of Std 1 (0 µg TX/mL) and A_S is the blank-corrected absorbance measured for the sample.

- Calculate the TEAC value of the samples using the following equation. Slope and intercept values are obtained from the standard curve.

$$\text{TEAC (µg TX/mL)} = \left(\frac{\% \text{ Inhibition of Sample} - \text{intercept}}{\text{slope}}\right)$$

When working with diluted samples the concentration values obtained must be multiplied by the dilution factor to obtain the TEAC (µg TX/mL) 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 UV/Vis: transparent 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) 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 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) 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 No analyte/activity is observed in the sample	Re-assay using different sample dilutions

STILL HAVING PROBLEMS?

Please, 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) Friday: 8.00 to 15.00 (CEST)

11. Additional information

BQC DMPD Assay Kit is an easy and quick (< 30 minutes) assay for determining TAC in food samples, beverages, and plant extracts.

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
KF01004	ORAC Antioxidant Capacity Assay Kit
KB03002	Lipid Peroxidation Assay Kit
KB03011	Superoxide Dismutase Activity 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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