Dynamic Test Kits for R&D and Quality Control

DPPH ANTIOXIDANT CAPACITY ASSAY KIT

KF01007

100/200 TESTS

96 well plate

BQCkit

A brand of Bio QuChem

Votre interlocuteur en France, Belgique, Luxembourg et Suisse :
LIBIOS
83, rue Edmond Michelet - 69490 Pontcharra Sur Turdine - France
Tél. : +33 (0)4 74 13 03 02 - Fax : +33 (0)4 74 05 28 25 –
Mail : info@libios.fr - www.libios.fr

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1.GENERAL INFORMATION

Please read this manual carefully before performing the assay.

PRECAUTIONS

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. Maintain order and cleanliness where dangerous products are used. It is recommended to use basic Personal Protective Equipment. For more information on the risks and preventative measures, check the MSDS available at bqckit.com.

Do not use after the expiring date. Store reagents as indicated on the section Materials on page 6.

TECHNICAL RECOMMENDATIONS

Keep enzymes, heat labile components and samples on ice. Let the components reach room temperature before use.

Invert the bottles a few times to ensure the reagents are well mixed before running the assay. Avoid foaming or bubbles when mixing or reconstituting components. Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.

Ensure plates are properly sealed or covered during incubation steps. Ensure complete removal of all solutions and buffers from tubes or plates during wash steps. Make sure you have the right type of plate for your detection method of choice.

Make sure the heat block/water bath and microplate reader are switched on.

Do not run the standard curve and the samples at different times and do not reuse the calculations of another day. Keep the standard and the samples on the assay for the same amount of time. It is recommended to use a multi-channel pip ette if possible.

2. TECHNICAL SPECIFICATIONS

Available sizes:

100 tests: 6 standard, 44 samples

200 tests: 6 standard, 94 samples

The calculations are just an estimation assuming that all the samples were tested the same day and that every standard and sample is tested on duplicate. Test number refers to total number of wells to be evaluated.

Volume of sample required:

20 µl/test

Types of sample compatible:

Foods, beverages and plant extracts.

Linear range:

100 – 500 µM

Type of detection:

Colorimetric (517 nm)

Sensitivity:

0.113 % Inhibition/TEAC (µM)

Time required for the assay:

15 min

3. MATERIALS

MATERIALS SUPPLIED

Store kit components as indicated below:

100 tests

Product	Nºbottles	Amount	Storage (before use)	Storage (after use)
Reagent A	1	30 ml	RT	RT
Reagent B	1	Powder	4 ºC	-
Standard	1	Powder	-20 ºC	-
96-well plate	-	1	-	-

Each vial of Reagent B is valid for 100 tests, discard the remaining solution afterusage.

200 tests

Product	Nºbottles	Amount	Storage (before use)	Storage (after use)
Reagent A	1	60 ml	RT	RT
Reagent B	2	Powder	4 ºC	-
Standard	2	Powder	-20 ºC	-
96-well plate	-	2	-	-

Each vial of Reagent B is valid for 100 tests, discard the remaining solution afterusage.

MATERIALS NEEDED BUT NOT SUPPLIED

Materials:

- Double distilled water (ddH2O) as MilliQ
- Pipettes and pipettetips
- 1.5 ml tubes

Instrumentation:

- Microcentrifuge
- Vortex mixer
- Colorimetric microplate reader – equipped with filter for OD 517 nm

4. 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 the 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 another foodstuff of plant and animal origin.

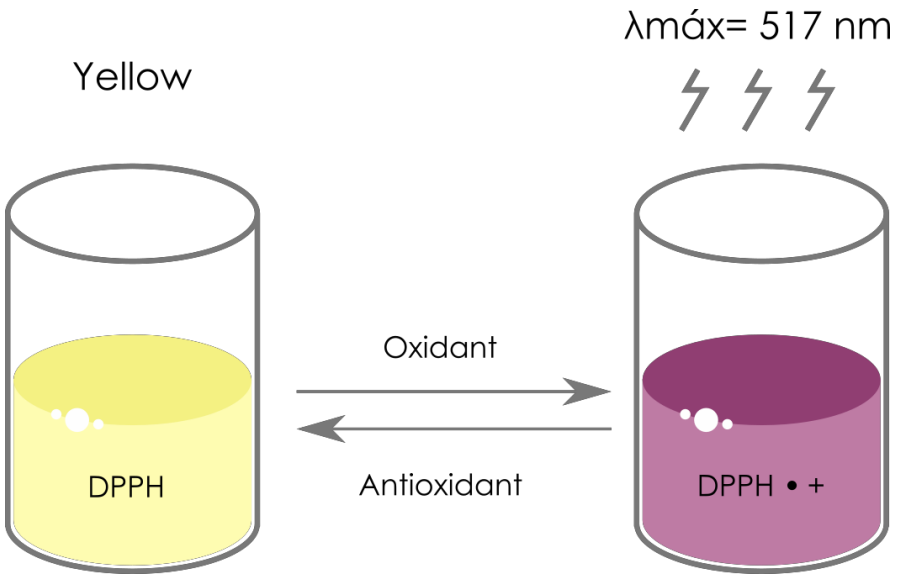
Antioxidative systems include antioxidative enzymes, that is, superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, and non-enzymatic substrates, such as glutathione, uric acid, lipoic acid, bilirubin, coenzyme Q, vitamin C (L-ascorbic acid), vitamin A (retinol), vitamin E (tocopherol), flavonoids, carotenoids, theine 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.

Total antioxidant capacity or TAC has been considered an overall parameter, which alterations have been linked to several conditions as infertility, obesity, cancer and neurodegenerative diseases.

BQC DPPH assay kit is an easy and highly reproducible assay to test TAC on single antioxidants in aqueous-organic solutions, on food and beverages

5. ASSAY PRINCIPLE

This kit measures the antioxidant activity of compounds that are able to transfer hydrogen atoms. The compound ($DPPH^{\bullet+}$) is a coloured and stable radical cation of purple colour which shows a maximum of absorbance at 517 nm. Antioxidant compounds, which are able to transfer an electron to $DPPH^{\bullet+}$, cause a discoloration of the solution. This reaction is rapid and proportional to the antioxidant capacity of the sample.



6. SAMPLE PREPARATION

BQCkit have tested the samples indicated below.

Sample	Preparation required	Dilution factor	Diluent	Long term storage
Fruitjuice	No	1:2	ddH_2O or Nutrition buffer*	-20ºC
Honey	Yes		ddH_2O or Nutrition buffer*	-20ºC
Wine	Yes	1:20	ddH_2O or Nutrition buffer*	-20ºC

*The use of a buffer preserves the antioxidant capacity of the samples over the use of ddH_2O, but it's the user's discretion to use the Nutrition buffer (0.1 M phosphate buffer pH 5.8, available at libios.fr)

Samples from abnormal or extreme experimental conditions may require a different dilution factor. For sample preparation instructions refer to the section Preparation protocols on page 10.

Is your sample is not included on this list? Contact us, please.

PREPARATION PROTOCOLS

Reagents required for sample preparation are not supplied. Take into account the sample volume required per test, refer to section Technical Specifications on page 5.

Honey:

1

2

3

Dilute honey to a concentration of 0.3 g/ml with warm 0.1 M phosphate buffer pH 5.8 (available at libios.fr as Nutrition buffer).	Vortex to mix	Let it incubate in a water bath until completely dissolved.

Total time required: 15 min
Additional notes: Maintain the temperature of the water bath and the buffer between 20-40 ºC.

Juice/Smoothie:

Depending on the texture of the juice/smoothie it might need to be filtered through a 0.2 µm membrane filter.

Total time required: 5 min

7. ASSAY PREPARATION

REAGENT PREPARATION

Reagents not included on this list are ready to use as supplied.

Solution B: This solution must be freshly prepared and discarded after usage. For sizes over 100 tests, BQCkit provides several bottles of Reagent B so the assay could be performed at different times (1 time per Reagent B bottle). Add the amount of Reagent A specified below and keep in the dark under magnetic stirring without heat application. Use within 30 minutes since preparation.

- 100 tests kit: Add 20 ml of Reagent A to each bottle of Reagent B that is going to be used immediately to obtain enough Solution B for 100 tests.
- 200 tests kit: Add 20 ml of Reagent A to each bottle of Reagent B that is going to be used immediately to obtain enough Solution B for 100 tests.

CAUTION: Once prepared, the solution B cannot be stored or reused at a different time, so the number of tests indicated need to be performed all at once for each Reagent B bottle.

STANDARD PREPARATION

Add exactly 2 ml of Reagent A to the standard vials that are going to be used immediately and mix well. Dilute standard by half in a 1 ml tube with Reagent A. For example: 500 µl standard
+ 500 µl Reagent A to a final volume of 1 ml. Prepare the calibration curve in 1 ml tubes as shown below.

	Standard (µl)	Reagent A (µl)	Concentration (µM)
1	0	100	0
2	10	90	100
3	20	80	200
4	30	70	300
5	40	60	400
6	50	50	500

Antioxidant capacity is expressed as TEAC (Trolox Equivalent Antioxidant Capacity).

PLATE SET UP

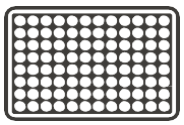
This scheme is just a recommendation on how to perform the assay. For optimal results, LIBIOS recommends running the standards and the samples at least for duplicate, but it is the user´s discretion to do so.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	C3	C3	C11	C11	C19	C19	C27	C27	C35	C35
B	S2	S2	C4	C4	C12	C12	C20	C20	C28	C28	C36	C36
C	S3	S3	C5	C5	C13	C13	C21	C21	C29	C29	C37	C37
D	S4	S4	C6	C6	C14	C14	C22	C22	C30	C30	C38	C38
E	S5	S5	C7	C7	C15	C15	C23	C23	C31	C31	C39	C39
F	S6	S6	C8	C8	C16	C16	C24	C24	C32	C32	C40	C40
G	C1	C1	C9	C9	C17	C17	C25	C25	C33	C33	C41	C41
H	C2	C2	C10	C10	C18	C18	C26	C26	C34	C34	C42	C42

8. ASSAY PROTOCOL

Before performing the assay, check the section Technical recommendations on page 3 to avoid any mistakes.

This assay should be performed with minimal light.

1 

Set up the plate design, you can use the BQCkit recommended set up (refer to section Plate set up on page 13) or use your own (refer to section Researcher notes on page 23)

2 

Add 20 µl of the sample or standard previously prepared (refer to sections Sample preparation on page 10 and Standard preparation on page 12)

3 

Add 200 µl of Solution B previously prepared (refer to section Reagent preparation on page 12) in each sample and standard well.

4 

Read the absorbance at 517 nm.

9. DATA ANALYSIS

ANALYSIS OF THE STANDARD

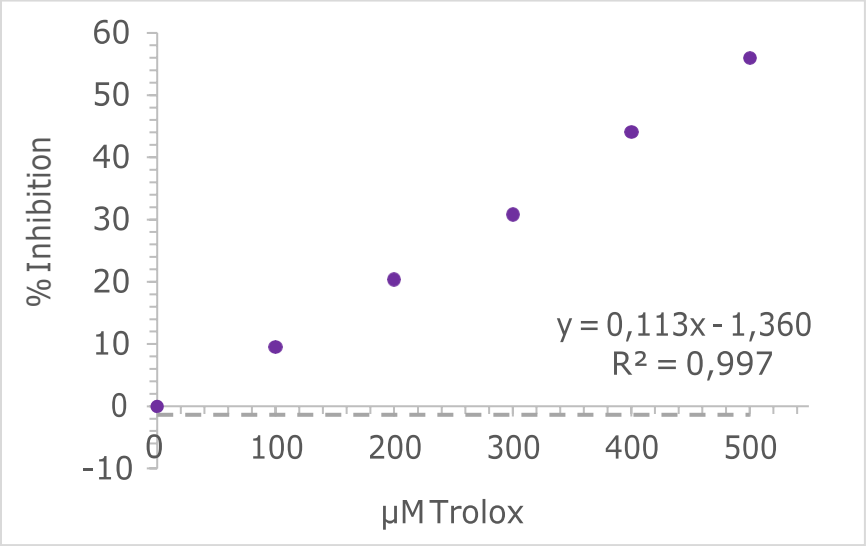
If the spectrophotometer or microplate reader was not zeroed with the blank, then average the blank values and subtract the average blank value from the standard and unknown sample values.

Calculate the percentage of inhibition of the radical $DPPH^{\bullet+}$ for each standard point with the following formula:

$$\% \text{ Inhibition} = \left(1 - \frac{A517\ Sn}{A517\ S1}\right) \cdot 100$$

Where A517 S1 is the DPPH•+ radical absorption without inhibition and A517 Sn is the DPPH•+ radical absorption of the correspondent standard.

Create a standard curve by plotting % inhibition (y-axis) vs. standard, µM Trolox (x-axis).



ANALYSIS OF THE SAMPLE

Determine the unknown sample concentration using the standard curve from the assayed sample value. Calculate the %inhibition from your samples as before, average the value for the replicates and then apply:

$$\text{TEAC } (\mu M) = \frac{(\%\ \text{inhibition-intercept})}{\text{slope}} * \text{dilution factor}$$

10. INTERFERING SUBSTANCES

To the best of our knowledge, no interfering substances have been founded.

11. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of the instrument
	Use of a different 96 well-plate	Colorimetric: Clear plates, Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use TCA precipitation protocol for deproteinization
	Cells/Tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substances in the sample	Check protocol for interfering substances

Lower/Higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (<5 µl) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/below the linear range	Concentrate/Dilute sample so it is within the linear range

12. RELATED PRODUCTS

More products available on libios.fr

Reference	Product
KF01001	DMPD Antioxidant Capacity Assay Kit
KF01002	ABTS Antioxidant Capacity Assay Kit
KF01003	FRAP Antioxidant Capacity Assay Kit
KF01004	ORAC Antioxidant Capacity Assay Kit
KF01005	CUPRAC Antioxidant Capacity Assay Kit

13. RESEARCHER NOTES

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14. 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, 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 Bioquochem´s gross negligence. Any and all liability of Bioquochem hereunder shall be limited to the amounts paid by the buyer for the 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 the arrival of the material at its destination.

Expiration date: 1 year from the date of deliver

Votre interlocuteur en France, Belgique, Luxembourg et Suisse :
LIBIOS
83, rue Edmond Michelet - 69490 Pontcharra Sur Turdine - France
Tél. : +33 (0)4 74 13 03 02 - Fax : +33 (0)4 74 05 28 25
Mail : info@libios.fr - www.libios.fr