LIBIOS

Dynamic Test Kits for R&D and Quality Control

FAST FRAP ANTIOXIDANT CAPACITY ASSAY KIT

KF01006

100/200/500 TESTS

96 well plate

BQCkit

A brand of BioQuoChem

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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 www.bqckit.com.

Do not use after the expiring date. Store reagents as indicated on the section Materials on page 5.

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 pipette if possible.

2. TECHNICAL SPECIFICATIONS

Available sizes:

100 tests: 9 standard, 41 samples

200 tests: 9 standard, 91 samples

500 tests: 9 standard, 241 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:

10 µl/test

Types of sample compatible:

Biological fluids and beverages.

Linear range:

7.5-250 µg/ml

Type of detection:

Colorimetric (593 nm)

Sensitivity:

0.01 (OD 593/FRAP µM)

Time required for the assay:

10 min

3. MATERIALS

MATERIALS SUPPLIED

Keep Reagent A tightly closed and avoid exposure to air. Never introduce pipette tips in the bottle, instead pour the necessary amount in a beaker container in case not all the tests are performed at once. To ensure stability, BQCkit provides several bottles of Reagent A, so as far as possible, use one bottle at a time. Store kit components as indicated below:

100 tests

Product	Nº bottles	Amount	Storage (before use)	Storage (after use)
Reagent A	5 bottles	4.5 ml	RT	RT
Standard	2 vials	Powder	RT	-
96-well plate	-	1	-	-

Each bottle of Reagent A is valid for 20 tests

200 tests

Product	Nº bottles	Amount	Storage (before use)	Storage (after use)
Reagent A	2 bottles	22 ml	RT	RT
Standard	2 vials	Powder	RT	-
96-well plate	-	1	-	-

Each bottle of Reagent A is valid for 100 tests

500 tests

Product	Nº bottles	Amount	Storage (before use)	Storage (after use)
Reagent A	3 bottles	30 ml	RT	RT
Standard	4 vials	Powder	RT	-
96-well plate	-	1	-	-

Each bottle of Reagent A is valid for 135 tests

MATERIALS NEEDED BUT NOT SUPPLIED

Materials:

- Double distilled water (ddH2O) as MilliQ
- Pipettes and pipette tips

Instrumentation:

- Microcentrifuge
- Vortex mixer
- Colorimetric microplate reader – equipped with filter for OD 593 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.

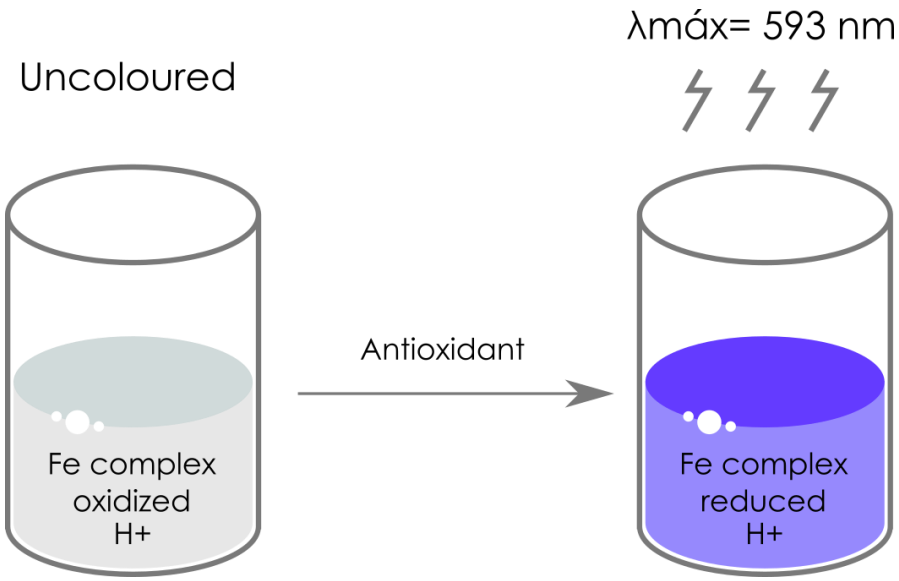
Antioxidative systems include antioxidative enzymes, that is, superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, and non-enzymatic substrates, such as glutathione, uric acid, lipoic acid, bilirubin, coenzyme Q, vitamin C (L-ascorbic acid), vitamin A (retinol), vitamin 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 FAST FRAP assay kit is a ready-to-use, easy and highly reproducible assay to test TAC on single antioxidants in aqueous solutions, added to plasma and on beverages with the outstanding feature of being faster than the FRAP classical assay.

5. ASSAY PRINCIPLE

This kit measures the antioxidant activity of compounds that are able to reduce the ferric complex. When the complex is at an acidic pH, in the presence of a suitable antioxidant solution, it is reduced, which shows a maximum of absorbance at 593 nm. 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
Plasma	No	-	-	-20 ºC
RPMI Culture medium + NAC	No	-	-	-20 ºC
DMEM Culture medium + NAC	No	-	-	-20 ºC
Gingseng juice	No	-	-	-20 ºC
Orange juice	No	-	-	-20 ºC
Pineapple juice	No	-	-	-20 ºC
Litchi juice	No	1:2	ddH_2O	-20 ºC
Apple juice	No	-	-	-20 ºC
Red berries smoothie	Yes	1:2	ddH_2O	-20 ºC
Kiwi smoothie	Yes	-	-	-20 ºC

Samples from abnormal or extreme experimental conditions may require a different dilution factor. For sample preparation instructions refer to the section preparation protocol, on page 9.

Is your sample is not included on this list? Check the BQCkit Testing Program and get a discount on your next order!

PREPARATION PROTOCOL

Reagents and materials required for sample preparation are not supplied. Take in account the sample volume required per test, refer to section Technical Specifications on page 4.

Smoothies:

1

Use a food processor to make the smoothie

2

Filter through a 0.2 µm membrane filter

3

Cool on ice to assay or freeze at -20ºC

Total time required: 15 min

7. ASSAY PREPARATION

REAGENT PREPARATION

Reagents in this kit are ready to use as supplied.

STANDARD PREPARATION

Add exactly 1 ml of ddH_2O to the standard vials that are going to be used immediately and mix well. Dilute standard 1:10 with ddH_2O. For example: 100 µl standard + 900 µl Reagent ddH_2O.

Prepare the calibration curve in 1.5 mL tubes as shown below.

	Standard (µl)	ddH2O (µl)	FRAP (µM)
1	0	100	0
2	2.5	97.5	100
3	5	95	200
4	7.5	92.5	300
5	10	90	400
6	12.5	87.5	500
7	15	85	600
8	17.5	82.5	700
9	20	80	800

Antioxidant activity is expressed as FRAP values (Ferric Reducing Ability of Plasma). These values are related to Fe^{2+} concentration. If preferred, Trolox, ascorbic acid and gallic acid can be used instead, but those are not supplied in this kit.

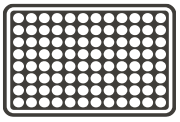
PLATE SET UP

This scheme is just a recommendation on how to perform the assay. For optimal results, BQCkit 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	S9	S9	C8	C8	C16	C16	C24	C24	C32	C32
B	S2	S2	C1	C1	C9	C9	C17	C17	C25	C25	C33	C33
C	S3	S3	C2	C2	C10	C10	C18	C18	C26	C26	C34	C34
D	S4	S4	C3	C3	C11	C11	C19	C19	C27	C27	C35	C35
E	S5	S5	C4	C4	C12	C12	C20	C20	C28	C28	C36	C36
F	S6	S6	C5	C5	C13	C13	C21	C21	C29	C29	C37	C37
G	S7	S7	C6	C6	C14	C14	C22	C22	C30	C30	C38	C38
H	S8	S8	C7	C7	C15	C15	C23	C23	C31	C31	C39	C39

S1-S9: Standard wells, C1-C39: Sample wells

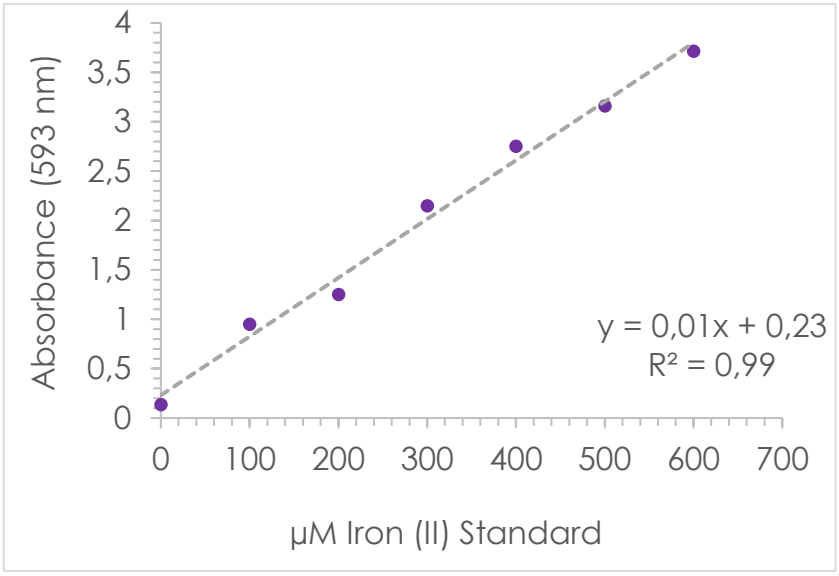
8. ASSAY PROTOCOL

1		Set up the plate design, you can use the BQCkit recommended set up (refer to section Plate set up on page 12) or use your own (refer to section Researcher notes on page 19)
2		Add 10 µl of the sample or standard previously prepared (refer to sections Sample preparation on page 9 and Standard preparation on page 11).
3		Add 220 µl of FRAP Working solution previously prepared (refer to section Reagent preparation on page 11) in each sample and standard well.
4		Mix for 4 minutes under continuous stirring
5		Read the absorbance at 593 nm

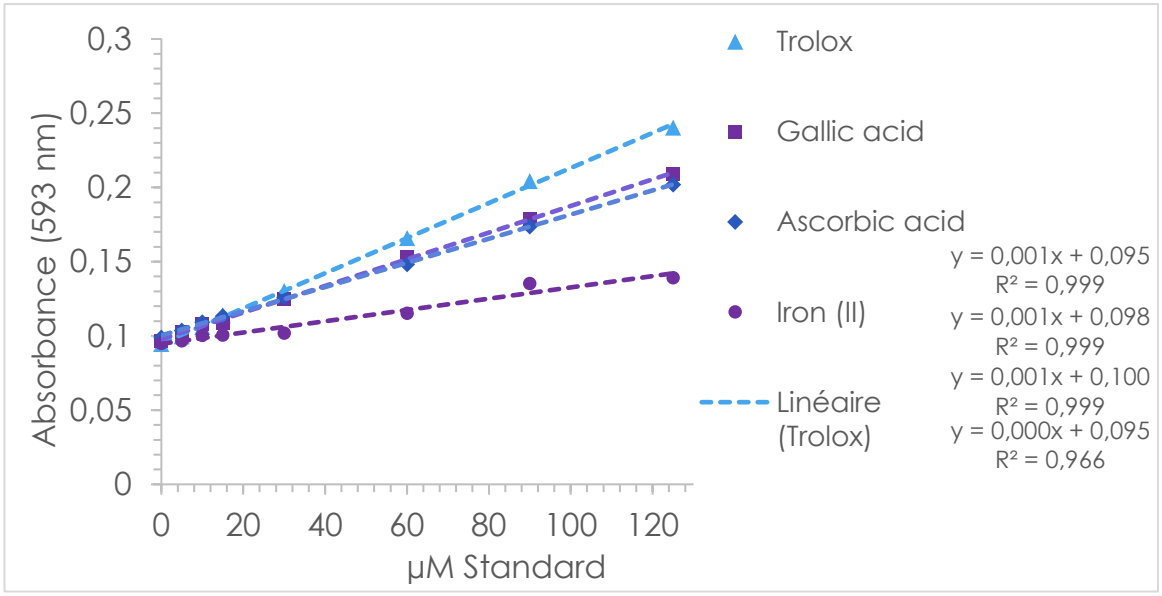
9. DATA ANALYSIS

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. Create a standard curve by plotting A 593 nm (y-axis) vs. standard, FRAP µM (x-axis).



Below, an example of the calibration curve with the four different antioxidant standards (up to 125 µM) that can be used with this kit can be seen.



ANALYSIS OF THE SAMPLE

Determine the unknown sample concentration using the standard curve from the assayed sample value. Average the OD for the replicates and then apply:

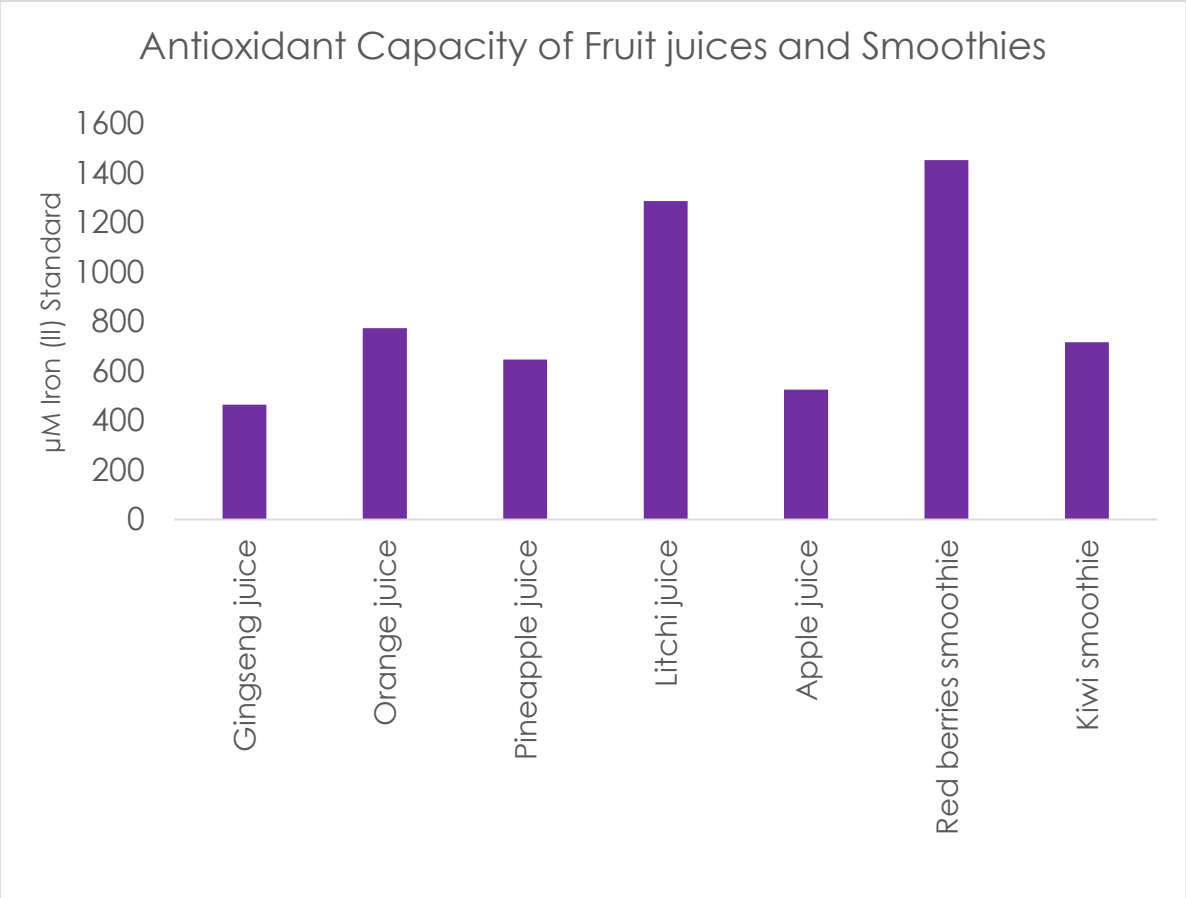
$$\text{FRAP } \mu M = \left(\frac{\text{OD 593 nm-intercept}}{\text{slope}}\right) * \text{dilution factor}$$

Usual values obtained on the samples:

Sample type	Range of values (FRAP µM)
Plasma	500-2000
Fruit Juices and Smoothies	400-1500

Those values are merely informative and can be affected by multiple factors.h

Some results obtained by BQCkit are shown below:



10. INTERFERERING SUBSTANCES

The following substances have been found to interfere with the assay:

- Non-antioxidant reducing substances
- Strong basic substances.

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 80ºC (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. RESEARCHER NOTES

	1	2	3	4	5	6	7	8	9	10	11	12
A												
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13. WARRANTIES AND LIMITATION OF LIABILITY

Our partner 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: 3 months from the date of delivery

Votre interlocuteur en France, Belgique, Luxembourg et Suisse :
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