LIBIOS
Dynamic Test Kits for R&D and Quality Control

FRAP Assay Kit

KF-01-003

500 tests (96 well plate)

BQCkit
A brand of BioQuoChem

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All chemicals should be handled with

- 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 FRAP Assay kit KF01003-500 tests contains:

Product	Quantity	Storage
FRAP Reagent A	1 bottle	RT
FRAP Reagent B	3 vials (powder)	RT
FRAP Reagent C	1 bottle (powder)	RT
FRAP Reagent D	1 bottle	RT
FRAP Standard	3 vials (powder)	RT

Assay Principle

Bioquochem FRAP assay kit is recommended for total antioxidant activity of single antioxidants in aqueous solution and added to plasma.

The assay described here measures the ferric reducing ability of plasma (FRAP). At low pH, when a ferric complex is reduced to the ferrous form (Fe^{2+}), an intense blue color with an absorption maximum at 593 nm develops.

This reaction is nonspecific and any half-reaction which has a less-positive redox potential, under reaction conditions, than the Fe^{3+}/Fe^{2+} complex half reaction will drive Fe^{3+} complex reduction. Acidic conditions favor reduction of the complex and, thereby, color development, showed that an antioxidant is present.

$$Fe^{3+}\text{-}C + AOH \rightarrow Fe^{2+}\text{-}C(blue)(\lambda max = 593\ nm)$$

Scheme 1. Reaction of Fe^{3+} complex with antioxidants (AOH)

Reagent Preparation

Solution B:

Add exactly 3.5 mL of ultrapure water in each vial of Reagent B and mix thoroughly. Once dissolved, keep refrigerated at -20°C.

Solution C:

Add exactly 12 mL of Reagent D in Reagent C vial. Once dissolved, keep refrigerated at 4°C.

FRAP working solution:

Prepare FRAP working solution just before use by mixing Reagents A, Solution B and Solution C (10:1:1). For example: 35 mL of Reagent A, 3.5 ml of Solution B and 3.5 mL of Solution C.

FRAP standard:

Add exactly 1 mL of ultrapure water in each Standard vial and mix thoroughly. Prepare standards immediately prior to the assay performed. Do not store the standard preparations. Dilute this solution 1:10 with ultrapure water.

Standard solutions:

Antioxidant activity is expressed as FRAP values (Ferric Reducing Ability of Plasma). These values are related to Fe^{2+} concentration.

Reagent Preparation

Prepare the calibration curve in 1 mL tubes as shown below in Table 1.

Table1. Reagent volumes needed to carry out the standard curve.

Standard [µL]	Diluent [µL]	FRAP [µM]
0	100	0
2.5	97.5	100
5	95	200
7.5	92.5	300
10	90	400
12.5	87.5	500
15	85	600
17.5	82.5	700
20	80	800

Assay Protocol

Sample preparation

Dilute your sample to an absorbance value corresponding to 500-600 µM of standard approximately.

Plasma samples do not usually need to be diluted.

Performing the assay

1. Add 10 µL of the sample or standard in each well.
2. Add 220 µL of FRAP working solution previously prepared (see Reagents Preparation) in each well.
3. Mix the mixture for 4 minutes under continuous stirring.
4. Read the absorbance at 593 nm.

Data Analysis

1. Zeroed the absorbance values:
2.

ΔA593 nm = **Δ**A593 nm sample/standard - A593 nm blank

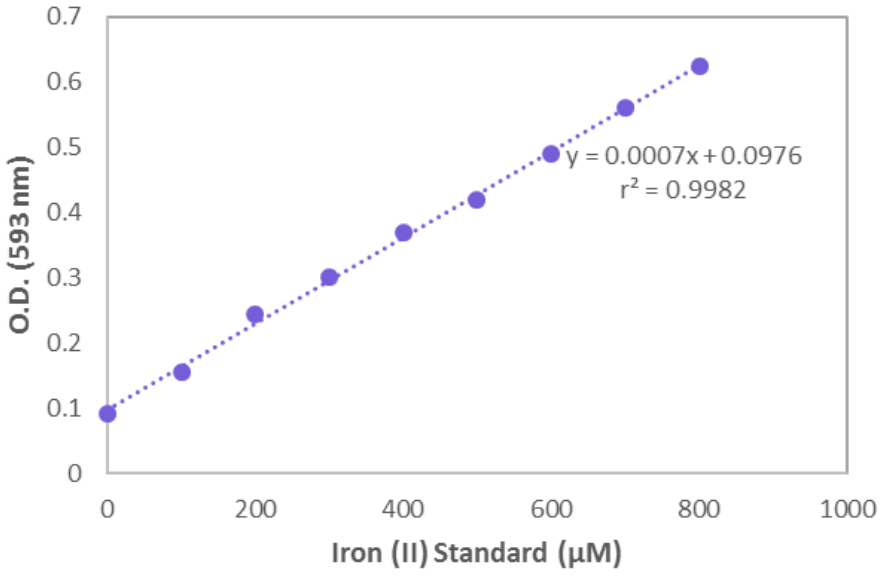
Where A593 nm sample/standard is the absorbance measured 4 minutes after the addition of antioxidants from samples or standards.

3. Plot the zeroed absorbance (ΔA593 nm) of standards as a function of their final concentrations (Table 1). See Figure 1 for a typical standard curve.

4. Calculate the FRAP value of the samples using the equation obtained from the linear regression of the standard curve substituted ΔA593 nm values for each sample.

FRAP (**μ**M) = (ΔA593 nm – intercept) / slope

Figure 1. Example of the standard representation



Data Analysis

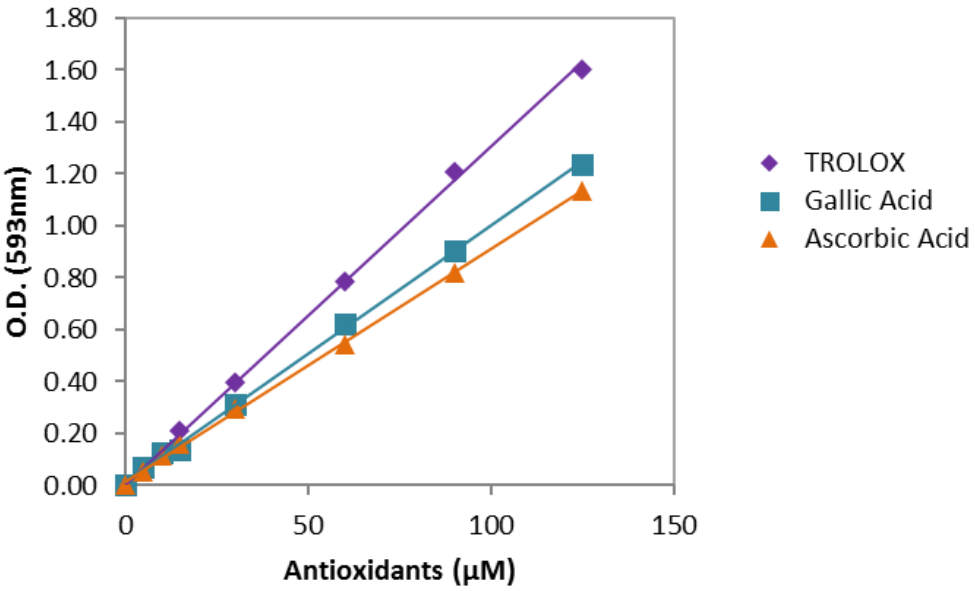


Figure 2. FRAP assay results for various antioxidants.

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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 arrival of the material at its destination.

Expiration date: 1 year from the date of delivery

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