FRAP Antioxidant Assay

(Cat. # BAQ066)

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.

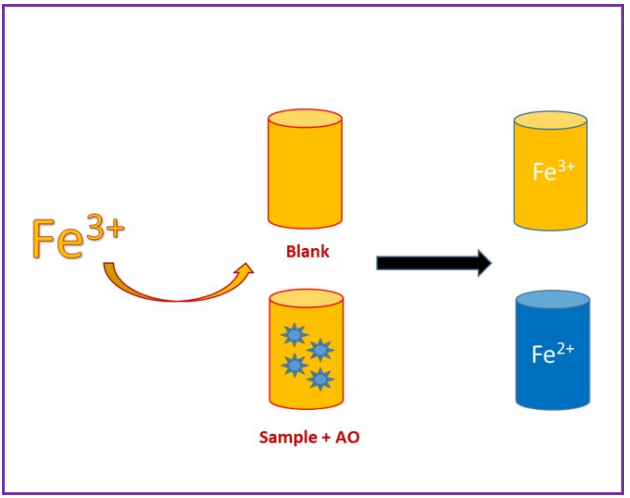
Antioxidative systems include antioxidative enzymes, that is, superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, and nonenzymatic substrates, such as glutathione, uric acid, lipoic acid, bilirubin, coenzyme Q, vitamin C (L-ascorbic acid), vitamin A (retinol), vitamin E (tocopherol), flavonoids, carotenoids, and others. Some biomolecules are also considered biologically active and clinically significant antioxidants, for example, transferrin, ferritin, lactoferrin, ceruloplasmin, hemopexin, haptoglobin, and uric acid.

G-Biosciences' 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.

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



ITEM(S) SUPPLIED

Description	500 tests (96 well plate)
FRAP Reagent A	1 bottle
FRAP Reagent B	3 vials (powder)
FRAP Reagent C	1 bottle (powder)
FRAP Reagent D	1 bottle
FRAP Standard	3 vials (powder)

STORAGE CONDITIONS

This kit is shipped at ambient temperature.. Store all the reagents as indicated on the labels. If stored and used as directed this kit is stable for 12 months.

ADDITIONAL ITEMS REQUIRED

- Spectrophotometer microplate reader that can measure at 593 nm
- 96 well microtiter plate for microplate assay.
- 1.5ml Tubes

PREPARATION BEFORE USE

Solution B:

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

Solution C:

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

FRAP working solution:

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

FRAP standard:

Add 1 mL of ultrapure water to each Standard vial and mix thoroughly. Prepare standards immediately prior to the assay being 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.

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

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

PROTOCOL

Sample preparation

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

Plasma samples do not usually need to be diluted.

Performing the assay

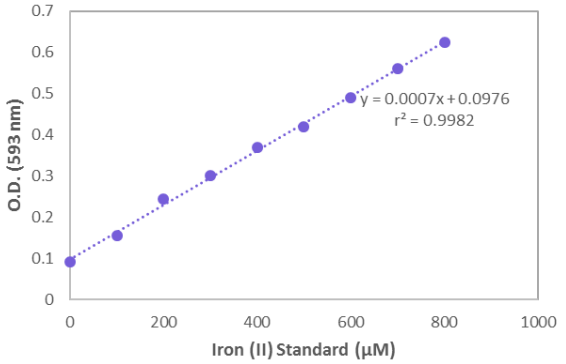
1. Add 10 µL of the sample or standard to each well.
2. Add 220 µL of FRAP working solution previously prepared to each well.
3. Mix the mixture for 4 minutes under continuous stirring.
4. Read the absorbance at 593 nm.

DATA ANALYSIS

Zero the absorbance values: ΔA593 nm = ΔA593 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.

Plot the zeroed absorbance (ΔA593 nm) of standards as a function of their final concentrations.

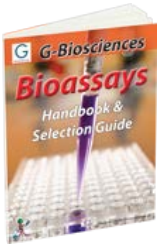


Calculate the FRAP value of the samples using the equation obtained from the linear regression of the standard curve substituted ΔA593 nm values for each sample.

$$\text{FRAP } (\mu M) = (\Delta A593 \text{ nm} - \text{intercept}) / \text{slope}$$

RELATED PRODUCTS

Download our Bioassays Handbook.



http://info2.gbiosciences.com/complete-bioassay-handbook

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