DPPH Antioxidant Assay

(Cat. # BAQ103, BAQ104)

INTRODUCTION

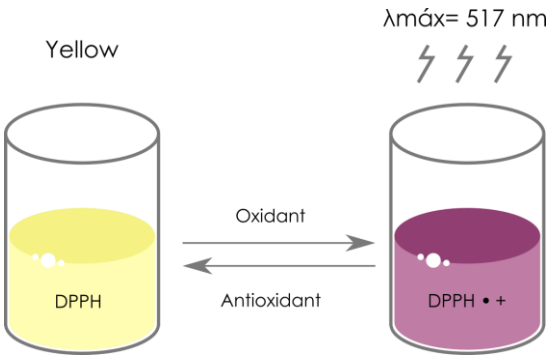
Antioxidant capacity is an overall ability of organisms or food to catch free radicals and prevent their harmful effect. Antioxidative effect includes protection of cells and cellular structures against the harmful effect of free radicals, especially oxygen and nitrogen. Substances with antioxidative 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.

Alterations in total antioxidant capacity or TAC have been linked to several conditions such as infertility, obesity, cancer and neurodegenerative diseases.

G-Biosciences, DPPH Antioxidant Assay is an easy and highly reproducible assay to test on single antioxidants in an aqueous organic solutions, food and beverages.

This kit measures the antioxidant activity of compounds that are able to transfer hydrogen atoms. The compound ($DPPH^{\bullet+}$) is a colored and stable radical cation of purple color 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.



ITEM(S) SUPPLIED

Description	Cat #: BAQ103 100 tests (96 well plate)	Cat #: BAQ104 200 tests (96 well plate)
DPPH Reagent A	30 ml	60 ml
DPPH Reagent B	1 bottle	2 bottles
DPPH Standard	1 vial	2 vials

STORAGE CONDITIONS

This kit is shipped with blue ice. 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 517 nm
- 96 well microtiter plate for microplate assay.
- 1.5 ml microfuge tubes.

SPECIFICATIONS

- The assay is available in 100 tests kit (Cat. # BAQ103) and 200 tests kit (Cat. # BAQ104) in a 96-microwell plate format.
- Colorimetric assay with linear detection range of 100- 500 µM.
- 0.113 % Inhibition/ Trolox Equivalent Antioxidant Capacity (TEAC) (µM)

IMPORTANT INFORMATION

- 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 mixed well 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.
- The assay should be performed in minimal light.
- Antioxidant capacity is expressed as Trolox Equivalent Antioxidant Capacity (TEAC). Trolox is used as standard.

PREPARATION BEFORE USE

DPPH Reagent B working solution

This solution must be freshly prepared and discarded after usage. For sizes of 100 tests 1 bottle of DPPH Reagent B containing powder is available and 2 bottles for 200 tests. Add 20 ml DPPH Reagent A per bottle of reagent B. This is enough for 100 tests in 96-well plate.

NOTE: *Once prepared DPPH Reagent B solution cannot be stored or reused at different time. Therefore for 100 tests kit, the tests need to be performed at one time, immediately after is reconstitution with* DPPH Reagent A*. For 200 tests kit, the assay can*

be performed at 2 different time points as 2 bottles of DPPH Reagent B are provided with the kit.

Standard preparation

Add 2 ml of DPPH Reagent A to the DPPH Standard and mix with help of a pipette. Dilute standard by half in a 1.5 ml microfuge tube with DPPH Reagent A. For example: 500 µl DPPH Standard + 500 µl DPPH Reagent A to a final volume of 1 ml. Prepare the calibration curve in 1.5 ml microfuge tubes as shown below.

Standard [µL]	Diluent (DPPH Reagent A) [µL]	Concentration [µM]
0	100	0
10	90	100
20	80	200
30	70	300
40	60	400
50	50	500

Table1

PROTOCOL

Sample preparation

- Sample dilution buffer is not supplied with kit. For food samples use 0.1 M phosphate buffer pH 5.8 or any other buffer that preserve the antioxidant capacity of the sample. Table2 gives the list of food samples that has been tested.
- **Honey sample**: Dilute honey to a concentration of 0.3 g/ml with warm 0.1 M phosphate buffer pH 5.8. Vortex to mix and incubate diluted honey sample in water bath for 15 minutes at 30°C until completely dissolved.
- **Juice/smoothie sample**: Depending on the texture of the juice/smoothie it might need to be filtered through a 0.2 µm membrane filter.

Sample	Preparation required	Dilution factor	Diluent	Long term storage
Fruit juice	No	1:2	0.1 M phosphate buffer pH 5.8 or double distilled water	-20°C
Honey	Yes		0.1 M phosphate buffer pH 5.8 or double distilled water	-20°C
Wine	Yes	1:20	0.1 M phosphate buffer pH 5.8 or double distilled water	-20°C

Performing the assay

1. Add 20 µl of the sample or standard to each well.
2. Add 200 µl of freshly prepared DPPH Reagent B working solution to each well with help of multichannel pipette and mix well.
3. Incubate the plate in dark for 3 to 5 minutes.

4. Read the absorbance at 517 nm.

DATA ANALYSIS

Analysis of the Standard

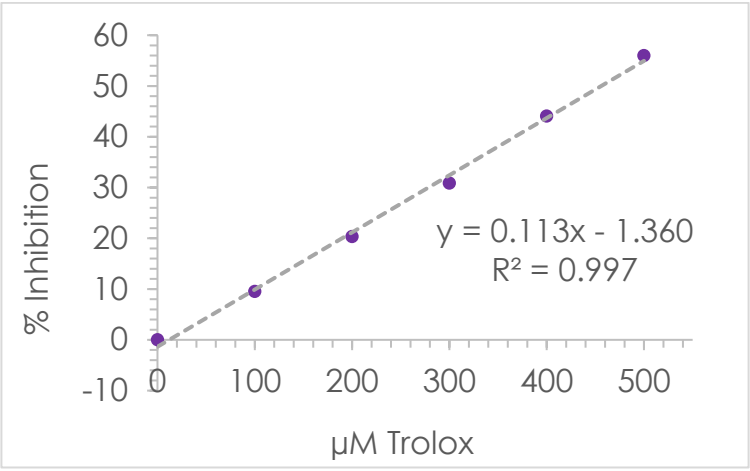
If the spectrophotometer or microplate reader was not zeroed with the blank, then average the blank values and subtract the average blank value from the standard and unknown sample values.

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:

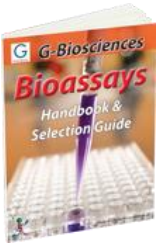
$$\text{TEAC } (\mu M) = \left(\frac{\%\ \text{inhibition-intercept}}{\text{slope}}\right) * \text{dilution factor}$$

TROUBLESHOOTING

Issue	Suggested reason	Possible solution
Assay not working	Use of ice-cold buffer	Bring buffers to room temperature before use for assay
	Plate read at incorrect wavelength	Check the wavelength and filter settings of the instrument
	Use of wrong 96-well plate	Use clear plates only.
Sample with erratic readings	Sample not deproteinized	Deproteinize samples with TCA or sulfosalicylic acid
	Cells and tissue samples not homogenized	Use Dounce homogenizer or increase the number of strokes
	Sample used after multiple freeze thaw cycle	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
Lower/Higher readings in samples or 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

RELATED PRODUCTS

Download our Bioassays Handbook.



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