DMPD Assay

(Cat. # BAQ065)

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.

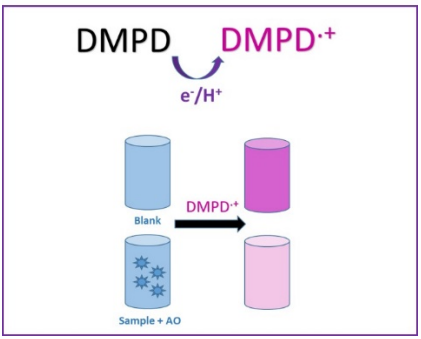
G-Biosciences' DMPD assay kit measures the antioxidant activity of compounds that are able to transfer hydrogen atoms.

When the compound N,N-dimethyl-p-phenylenediamine (DMPD) is in the presence of a suitable oxidant solution, a colored radical cation is formed (DMPD•+). Antioxidant compounds, which can transfer a hydrogen atom to DMPD•+, cause a decoloration of the solution.

In our assay a solution of DMPD at an acidic pH and in the presence of a suitable oxidant solution, can form a stable and colored radical cation (DMPD•+) which shows a maximum of absorbance at 553 nm. Antioxidant compounds which can transfer a hydrogen atom to DMPD•+ quench the color and produce a decoloration of the solution which is proportional to their amount. This reaction is rapid and the end point, which is stable, is taken as a measure of the antioxidative efficiency.

DMPD (uncolored) + Oxidant + H+ → DMPD• + (purple)(λmax= 553 nm)

DMPD• + (purple) + AOH → DMPD(uncolored) + AO



ITEM(S) SUPPLIED

Description	200 tests (96 well plate)
DMPD Reagent A	1 vial (powder)
DMPD Reagent B*	1 vial
DMPD Reagent C	2 vials
DMPD Reagent D	2 bottles
DMPD Standard*	1 vial (powder)

STORAGE CONDITIONS

The kit is supplied on 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 553 nm
- 96 well microtiter plate for microplate assay.
- 1.5ml Tubes

PREPARATION BEFORE USE

Sample Preparation

If your sample is colored, dilute it to an absorbance value lower than the measure A_0.

Allow the reagents to reach room temperature before use.

DMPD Reagent A

Add 1mL of ddH_2O to the DMPD Reagent A vial and mix thoroughly. This solution should be kept at -20°C for up to 1 month.

DMPD•+ solution

To each DMPD Reagent D bottle, add 300µl of DMPD Reagent A and 60µl of DMPD Reagent B. Mix well and let stand at room temperature for 10 minutes to allow the radical to perform. This solution should be freshly prepared.

Standard curve preparation:

Add 1 mL of DMPD Reagent C to the DMPD Standard vial. This solution should be kept at -20°C for up to 1 month. Then dilute the standard solution 10 times in a 1.5 mL vial (not provided) to perform the calibration curve. This solution should be freshly prepared.

Antioxidant activity is expressed as TEAC (Trolox equivalent antioxidant capacity). For this purpose, use the 1:10 diluted standard prepared above.

Prepare calibration curves in 1.5 mL tubes as shown below.

Sample	Reagent C [µL]	Diluted Standard [µL]	TEAC [µg/mL]
S1 (Blank)	500	0	0
S2	485	15	25
S3	465	35	50
S4	433	67	100
S5	416	84	125
S6	400	100	150
S7	366	134	200
S8	333	167	250

PROTOCOL

- If the antioxidant activity in the samples is not known or if it is expected to be beyond the range of the standard curve, it is recommended to assay the samples at several dilutions.
- For optimal results, it is recommended to run the standards and the samples for duplicate, but it is the user´s discretion to do so.

1. Add 20 µL of the sample or standard to each well.
2. Add 280 µL of DMPD•+ solution to each well.
3. Mix at room temperature for 10 minutes under continuous stirring.
4. Read the absorbance at 553 nm.

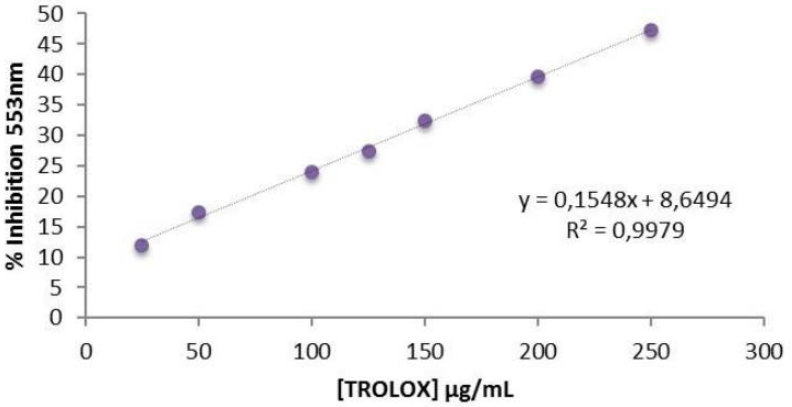
DATA ANALYSIS

Calculate the absorbance at 553 nm as percentage of the absorbance of the uninhibited radical cation solution (Blank) according to the equation:

$$\text{Inhibition of } A_{553}\ (\%) = (1-(A_f/A_0)) \times 100$$

Where A_0 is the absorbance of uninhibited radical cation and A_f is, the absorbance measured 10 min after the addition of antioxidant samples.

Plot the inhibition of standards as a function of their final concentrations.

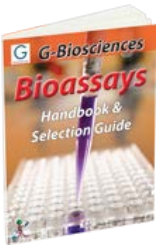


Calculate the TEAC value of the samples using the equation obtained from the linear regression of the standard curve substituted inhibition percentage values for each sample.

TEAC (µg/ ml) = (sample inhibition A553 - intercept) / slope

RELATED PRODUCTS

Download our Bioassays Handbook.



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