EZ-DPPH Antioxidant Assay Kit

Oxidative Stress Assay Kit

Cat. No. DG-DPH400

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

DoGenBio Co., Ltd.

▪ Product Description

Antioxidant ability plays an important role in human diseases and health problems. Substances with antioxidant ability are called antioxidants. Antioxidants are generally found in plants and animals such as fruits and vegetables. Consuming foods rich in antioxidants can reduce the risk of various diseases including cardiovascular disease and cancer.

EZ-DPPH Antioxidant Assay kit is a quick and simple method for evaluating antioxidant capacity using DPPH, known as a stable free radical. DPPH, which is purple, is reduced by antioxidants to form DPPH•H, which shows a pale yellow color and is measured colorimetric at 517 nm.

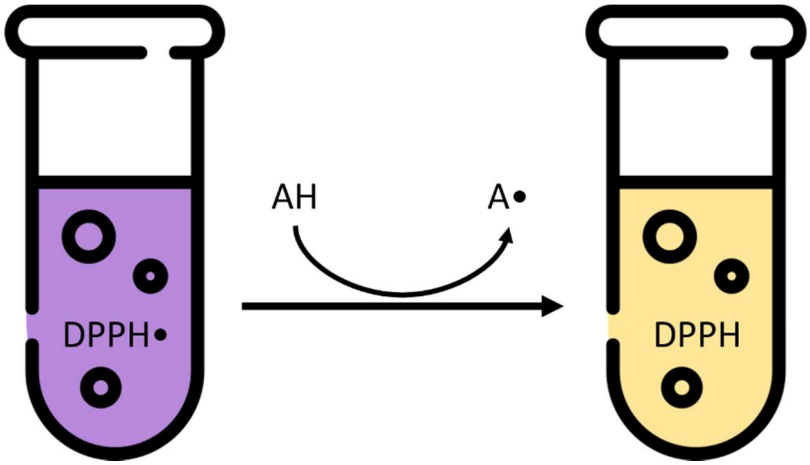


Fig. Reduction of free radical DPPH by antioxidants.

▪ Kit Contents and Storage Conditions

Component	400 assay	Storage
DPPH Reagent	4 vials	4 ℃, 1 year
DPPH Assay Buffer	20 ㎖ X 2	
Trolox Standard (1 mg)	4 vials	

* This product is for research purposes only and should not be used for human consumption or diagnostic purposes.
* The DPPH Assay Buffer should be allowed to reach room temperature before use.
* The ABTS Reagent should be prepared immediately before use and cannot be reused after use.

T. 070-7727-0456 F. 02-855-1550 www.dogenbio.com

▪ Preparation of Reagent

Solution	Preparation	Storage and Stability
DPPH Reagent	Add 1.1 ㎖ of ethanol and vortexing for 1 minute to completely dissolve. Once dissolved, add the DPPH Reagent to 9 ㎖ of ethanol to prepare the DPPH Reagent Solution.	DPPH Reagent should be used within 24 hours after adding ethanol. Prepare just before use and keep away from light.
Trolox Standard	Add 1 ㎖ of ethanol and mix well while gently inverting. Prepare just before use.	Use Trolox solution within 24 hours after adding ethanol.

***** Ethanol (anhydrous) and Proteinase K (e.g., Merck, P2308) used in this kit must be purchased separately.

* This protocol is optimized for use with a 96-well plate for experiments/measurements.

* Ensure that the DPPH Reagent is completely dissolved before use.

▪ General Protocol

Sample preparation

Note

• The method and amount of sample extraction may vary, and as a result, the dilution factor may also differ.

- Soft samples (e.g., fruits, vegetables, etc.): Grind or crush the sample, then extract before use.
- Other solid samples (e.g., seeds, roots, dried plant materials, etc.): Extract using homogenization or sonication before use.

• When measuring the concentration of all samples, the O.D. value should fall within the range of the Standard Curve. Therefore, prepare the samples at multiple concentrations for the experiment.

• For heat-sensitive samples, conduct the experiment on ice.

• If the sample contains EDTA (ethylenediaminetetraacetic acid), measurements may not be possible.

• Accurate measurements may be difficult if the sample contains particles or has color.

1. Serum

① Add 285 ㎕ of serum and 15 ㎕ of Proteinase K (20 mg/㎖) to a microtube.

② Incubate at 37°C for 45 minutes, then further incubate at 90°C for 10 minutes.

③ Centrifuge at 4°C, 12,000 x g for 60 minutes and use only the supernatant.

2. Fruits, vegetables, other foods and plants

① Add 500 ㎕ of DPPH Assay Buffer to 100 ㎎ of the sample, vortex thoroughly, then centrifuge at 10,000 x g for 10 minutes. Use the supernatant.

② For more accurate measurements, prepare samples at multiple concentrations using DPPH Assay Buffer.

3. Liquid Sample (Beverages including juices, wines, teas and others)

① Filter the sample using a 0.2 ㎛ filter and use it immediately.

② For accurate measurements, prepare samples of multiple concentrations using DPPH Assay Buffer.

③

Standard preparation

Dispense 0, 40, 80, 120, 160, 200 ㎕ of 1mM Trolox Standard Solution into 1.5 ㎖ microtubes. Add ethanol to adjust the final volume to 400 ㎕.

Standard No.	1mM Trolox Standard Solution	Ethanol	Final volume	Trolox Conc. (㎍/mL)
1	0 ㎕	400 ㎕	400 ㎕	0
2	40 ㎕	360 ㎕	400 ㎕	25
3	80 ㎕	320 ㎕	400 ㎕	50
4	120 ㎕	280 ㎕	400 ㎕	75
5	160 ㎕	240 ㎕	400 ㎕	100
6	200 ㎕	200 ㎕	400 ㎕	125

* It is recommended that the standard be measured for each experiment.

DPPH Radical Scavenging Activity Assay

: Trolox Equivalent Antioxidant Capacity (TEAC) is expressed as the EC_{50}* value for the sample. (EC_{50}*: concentration at which 50% of DPPH radicals are eliminated)

	Trolox Standard	Sample	Sample Blank	Blank 1	Blank 2
Trolox Standard Solution	20 ㎕				
Sample		20 ㎕	20 ㎕		
Solvent				20 ㎕	20 ㎕
Ethanol			100 ㎕		100 ㎕
Assay Buffer	80 ㎕	80 ㎕	80 ㎕	80 ㎕	80 ㎕
DPPH Reagent Solution	100 ㎕	100 ㎕		100 ㎕	

* Blank 1 : without antioxidant,
Blank 2 : ethanol blank,
Sample Blank : sample background blank.

* If the color of the sample is dark, compensate for the absorbance value of the sample color using a sample blank.

* DPPH Reagent Solution starts reacting the moment it is added, so add it at the very end to start the reaction.

* For accurate measurements, prepare standards and samples in duplicate or more according to concentration.

① Add 20 ㎕ of Trolox Standard, Sample, and Sample Blank at each concentration to each well.

② Add 20 ㎕ of ethanol to Blank 1 and Blank 2.

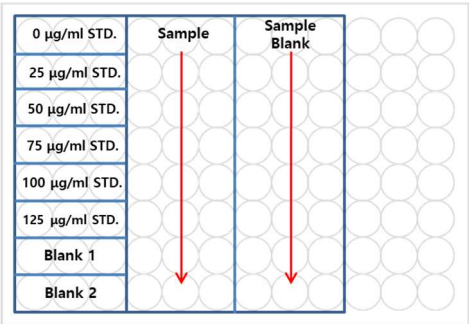
③ Add 80 ㎕ of Assay Buffer to each well.

④ Add 180 ㎕ of Ethanol to the Blank 2 and Sample Blank.

⑤ Add 100 ㎕ of DPPH Reagent Solution to Trolox Standard, Sample, and Blank 1, then mix well using a plate shaker and pipette.

⑥ Incubate the plate at room temperature (25°C), protected from light, for 10 minutes.

⑦ After the reaction, measure the absorbance at 517 nm using a plate reader.



▪ Calculation

All results should be prepared in duplicate or more, and calculations should be based on the average values.

✓ Calculation Example

① Calculate the average of the Sample and Sample Blank values.

반응농도	Sample	
ug/mL	Sample	Sample BLK
125	0.214	0.032
100	0.277	0.032
75	0.320	0.034
50	0.360	0.032
25	0.409	0.033
0	0.487	0.032
BLK1	0.661	0.000
BLK2	0.031	

② Subtract the Sample Blank value from the corresponding concentration in the Sample to obtain **A**, and subtract the Blank 2 value from Blank 1 to calculate **B**.

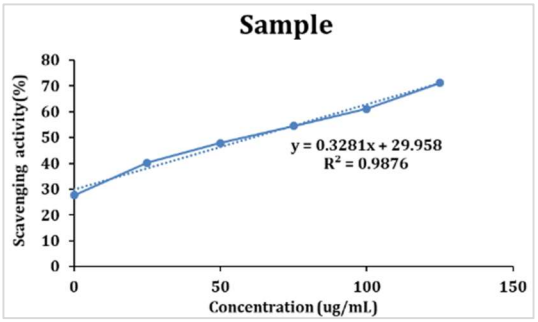
반응농도	Sample			
ug/mL	Sample	Sample BLK	A	SA(%)
125	0.214	0.032	=T28-U28	71.2
100	0.277	0.032	0.245	61.2
75	0.320	0.034	0.286	54.6
50	0.360	0.032	0.328	47.9
25	0.409	0.033	0.376	40.3
0	0.487	0.032	0.456	27.7
Blank1	0.661	0.000	B	
Blank2	0.031		0.630	

반응농도	Sample			
ug/mL	Sample	Sample BLK	A	SA(%)
125	0.214	0.032	0.181	71.2
100	0.277	0.032	0.245	61.2
75	0.320	0.034	0.286	54.6
50	0.360	0.032	0.328	47.9
25	0.409	0.033	0.376	40.3
0	0.487	0.032	0.456	27.7
Blank1	0.661	0.000	B	
Blank2	0.031		=T34-T35	

- To calculate the Scavenging activity (SA, %), use the values of A and B obtained previously and apply the following formula:
- **SA (%) : 100-(A/B)*100**

반응농도	Sample			
ug/mL	Sample	Sample BLK	A	SA(%)
125	0.214	0.032	=100-(V28/V35)*100	
100	0.277	0.032	0.245	61.2
75	0.320	0.034	0.286	54.6
50	0.360	0.032	0.328	47.9
25	0.409	0.033	0.376	40.3
0	0.487	0.032	0.456	27.7
Blank1	0.661	0.000	B	
Blank2	0.031		0.630	

③ Plot a scatter graph using the concentration values of the Sample and the SA (%) values, and display the equation and R^2 value using the trendline.



T. 070-7727-0456 F. 02-855-1550 www.dogenbio.com

✓ **Total Trolox Equivalents Antioxidant Capacity (TEAC)**

- EC_{50} can be determined by substituting 50 for y in the equation y = ax + b from the scavenging activity (%) graph, allowing calculation of the Trolox concentration required to scavenge 50% of ABTS radicals.
- For the Sample, measure the concentration values and absorbance values, then plot the scavenging activity (%) graph. From this, you can substitute the values to calculate the EC_{50} of the sample.
- TEAC is calculated using the formula below:

Antioxidant Capacity (TEAC): Trolox EC_{50} / Sample EC_{50}

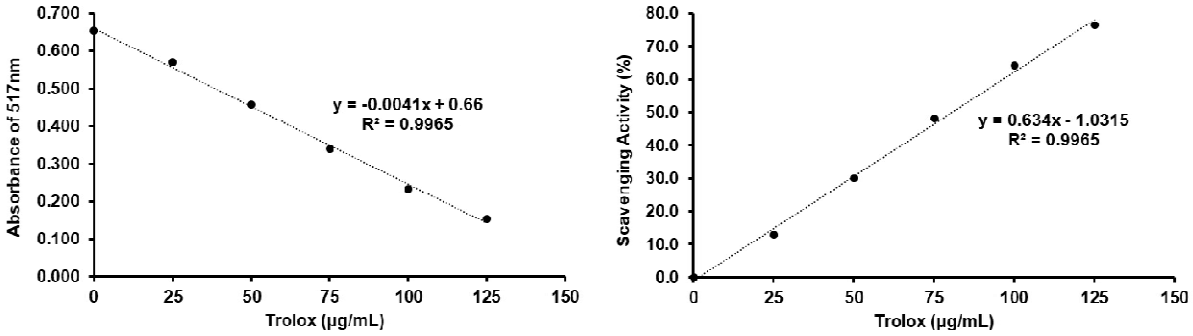


Fig. Trolox standard curve. Assay was performed following the kit protocol.

▪ Related Product

	Products	Catalog No.	Assay
Oxidative Stress Assay Kit	EZ-Superoxide Dismutase (SOD) Assay Kit (Colorimetric)	DG-SOD400	400 Assay
	EZ-Glutathione Assay Kit (Colorimetric)	DG-GLU200	200 Assay
	EZ-Catalase Assay Kit (Fluorometric/Colorimetric)	DG-CAT400	400 Assay
	EZ-Hydrogen peroxide/Peroxidase Assay Kit (Fluorometric/Colorimetric)	DG-PER500	500 Assay
	EZ-Lipid Peroxidation (TBARS) Assay Kit (Colorimetric)	DG-TBA200	200 Assay
	EZ-Total Antioxidant Capacity (TAC) Assay Kit (Colorimetric)	DG-TAC200	200 Assay
	EZ-DPPH Antioxidant Assay Kit (Colorimetric)	DG-DPH400	400 Assay
Metabolism Assay Kit	EZ-Lactate Assay Kit (Colorimetric)	DG-LAC100	100 Assay
	EZ-Acetylcholinesterase Assay Kit (Colorimetric)	DG-ACE100	100 Assay
	EZ-Ascorbic Acid Assay Kit (Colorimetric)	DG-ASC100	100 Assay
	EZ-ATP Assay Kit (Fluorometric/Colorimetric)	DG-ATP100	100 Assay
	EZ-Free Fatty Acid Assay Kit (Fluorometric/Colorimetric)	DG-FFA100	100 Assay
	EZ-Free Glycerol Assay Kit (Fluorometric/Colorimetric)	DG-FGC100	100 Assay
	EZ-Glucose Assay Kit (Fluorometric/Colorimetric)	DG-GCS100	100 Assay
	EZ-HDL, LDL/VDL Assay Kit (Fluorometric/Colorimetric)	DG-CHO100	100 Assay
	EZ-Total Cholesterol Assay Kit (Fluorometric/Colorimetric)	DG-TSC100	100 Assay
	EZ-Triglyceride Quantification Assay Kit (Fluorometric/Colorimetric)	DG-TGC100	100 Assay
	EZ-Nitric Oxide Assay Kit (Colorimetric)	DG-NO500	500 Assay