EZ-ABTS Antioxidant Assay Kit

Oxidative Stress Assay Kit

Cat. No. DG-ABT400

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

▪ Product Description

The ABTS assay is based on the principle that the ABTS+ cation radical, generated by reacting with potassium persulfate, is reduced by antioxidants. This method has the advantage of being less affected by pH compared to another antioxidant assay, the DPPH assay, which follows the same principle of radical scavenging.

Although both the ABTS and DPPH assays rely on the same principle of radical neutralization by antioxidants, the results may differ depending on the degree of interaction between the substrate and the reacting substance. Therefore, for accurate measurements, it is recommended to use both the ABTS assay (DG-ABT400, DoGENBio) and the DPPH assay (DG-DPH400, DoGENBio) together.

The EZ-ABTS Antioxidant Assay Kit utilizes the characteristic of ABTS, which appears turquoise in its cationic form and turns colorless when reacted with antioxidants and measures this color change colorimetrically at 732 nm.

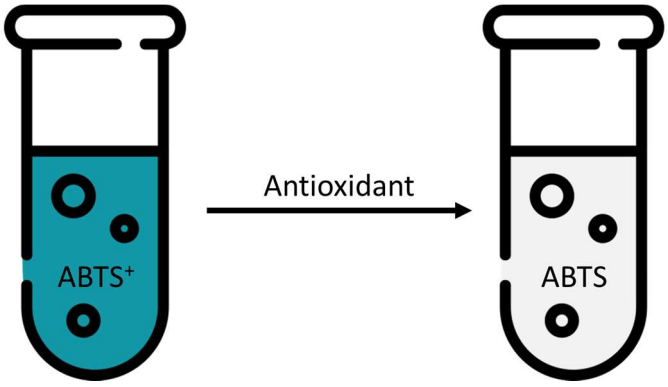

Fig. Reduction of radical ABTS by antioxidants.

▪ Kit Contents and Storage Conditions

Component	400 assay	Storage
ABTS Reagent	4 vials	4 ℃, 1 years
2X Assay Buffer	25 ㎖ 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 2X 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.

▪ Preparation of Reagent

Solution	Preparation	Storage and Stability
2X Assay Buffer	Prepare by mixing 11 ㎖ of 2X Assay Buffer and 11 ㎖ of distilled water (D.W). * For 1 plate of 96-well.	-
ABTS Reagent	Add 600 ㎕ of 1X Assay Buffer and dissolve completely. Once dissolved, add the ABTS Reagent to 20 ㎖ of 1X Assay Buffer to prepare the ABTS Reagent Solution.	The ABTS Reagent should be used within 24 hours after adding the Assay Buffer. Prepare it before use and protect the prepared solution from light before use.
Trolox Standard	Add 1 ㎖ of ethanol and mix well by gently inverting.	Use the 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 ABTS 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 1X 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 1X Assay Buffer.

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

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

② For more accurate measurements, prepare samples at multiple concentrations using 1X Assay Buffer.

Standard preparation

Dispense 0, 40, 80, 120, 160 ㎕ 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

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

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ABTS 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 ㎕		
Ethanol				20 ㎕	20 ㎕
1X Assay Buffer			180 ㎕		180 ㎕
ABTS Reagent Solution	180 ㎕	180 ㎕		180 ㎕	

* 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.

* ABTS 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 to each well according to their concentrations.

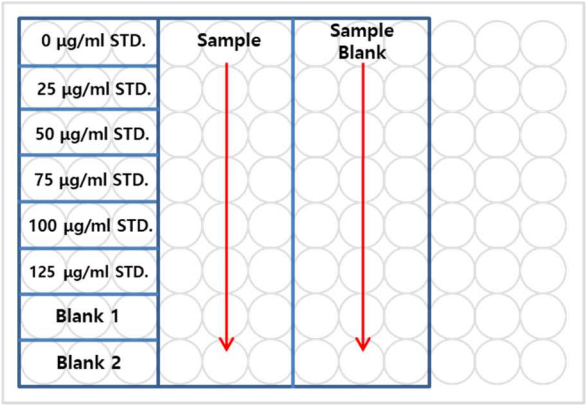
② Add 20 ㎕ of ethanol to Blank 1 and Blank 2.

③ Add 180 µL of 1X Assay Buffer to the Blank 2 and Sample Blank.

④ Add 180 ㎕ of ABTS Reagent Solution to Trolox Standard, Sample, and Blank 1, then mix well using a plate shaker and pipette.

⑤ Incubate the plate at room temperature (25°C), protected from light, for 10 minutes.

⑥ After the reaction, measure the absorbance at 732 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.

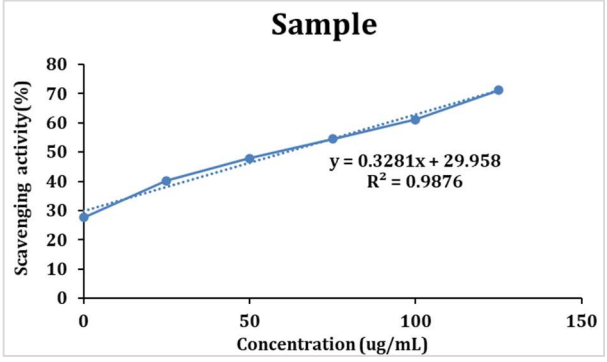
반응농도	Sample			
ug/mL	Sample	Sample BLK	A	SA(%)
125	0.214	0.032	=T28-U28	71.2
100	0.277	0.032	0.245	61.2
75	0.320	0.034	0.286	54.6
50	0.360	0.032	0.328	47.9
25	0.409	0.033	0.376	40.3
0	0.487	0.032	0.456	27.7
Blank1	0.661	0.000	B	
Blank2	0.031		0.630	

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.



✓ **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.
- The scavenging activity (%) of the sample can be determined by measuring absorbance values at different concentrations and calculating the EC_{50} using the resulting graph.
- TEAC is calculated using the formula below:

TEAC = Trolox EC_{50} / Sample EC_{50}

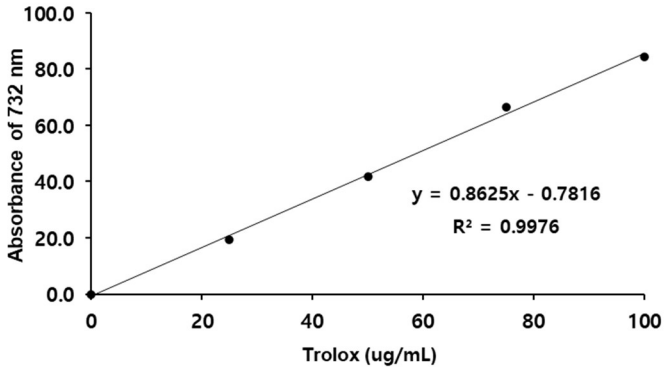


Fig. Trolox standard curve. Assay was performed following the kit protocol.

▪ 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
	EZ-Glutathione Peroxidase Assay Kit (Colorimetric)	DG-GPX100	100 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
	EZ-Total Collagen Assay Kit (Colorimetric)	DG-COL100	100 Assay
	EZ-Ethanol Assay Kit (Colorimetric)	DG-ETH100	100 Assay