

EZ-Total Antioxidant Capacity (TAC) Assay Kit

Oxidative Stress Assay Kit

Cat. No. DG-TAC200

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

▪ Product Description

Antioxidants play a crucial role in scavenging free radicals and other potential toxic oxidative species or preventing their formation. Antioxidants can be broadly classified into three categories: enzymes (such as GSH reductase, catalase, peroxidase), small molecules (such as ascorbate, uric acid, GSH, vitamin E), and proteins (such as albumin, transferrin).

Since antioxidants have varying reducing capacities depending on their characteristics, it is difficult to make direct comparisons, so standard substances are used. Among them, Trolox is widely used as a standard antioxidant, and all other antioxidants can be compared and measured relative to Trolox equivalents.

The EZ-Total Antioxidant Capacity (TAC) Assay kit takes advantage of the fact that most antioxidants are in their reduced form and measures the ability of antioxidants to reduce Cu^{2+} to Cu^{+} through a colorimetric method, with absorbance measured at 450 nm.

▪ Kit Contents and Storage Conditions

Component	200 assay	Storage
Trolox Standard (10 mM)	2 vial	4 °C
Copper Reagent	20 mL	4 °C
Reaction buffer	20 mL	4 °C

* This product is for research use only and is not intended for human or diagnostic use.

* In terms of the number of tests that can be performed with this product, 100 assays means that it provides reagents that can process 100 wells based on 1 well of a 96 well plate. Among these, considering standard, blank, duplication processing per sample, etc., the actual number of samples that can be tested is in the range of 20 to 40 samples. Review the product instructions carefully and determine the number of kits required considering the characteristics of the sample you wish to test.

▪ Preparation of Reagent

Solution	Preparation	Storage and Stability
Trolox Standard	Add 1 mL of ethanol and gently invert to mix thoroughly. Prepare it just before use and <u>do not vortex.</u>	Use the Trolox Standard Solution within 24 hours.

* Do not leave the Copper Reagent and Reaction Buffer at room temperature for more than 1 hour.

* Measurement may not be possible if the sample contains EDTA.

* For accurate standard curves, it is recommended to use ethanol with a concentration of 95% or higher for the Trolox standard.

▪ General Protocol

1. Standard preparation

Add 2, 4, 6, 8, and 10 μL of the 10 mM Trolox Standard Solution into 1.5 mL microcentrifuge tubes, and then add dH_2O to adjust the final volume to 100 μL .

Standard No.	Volume of 10 mM Trolox Standard Solution	Volume of dH_2O	Final standard volume in tube	Final standard Trolox Conc. (mM)
1	0 μL	100 μL	100 μL	0
2	2 μL	98 μL	100 μL	0.2
3	4 μL	96 μL	100 μL	0.4
4	6 μL	94 μL	100 μL	0.6
5	8 μL	92 μL	100 μL	0.8
6	10 μL	90 μL	100 μL	1.0

* It is recommended to measure the standard with each experiment.

2. Standard preparation

- All samples should be handled on ice. Prepare samples within 30 minutes to avoid effects from oxygen, light, or heat.
- **Urine** : Dilute with PBS (pH 7.0) for analysis.
- **Plasma** : Analyze directly without preprocessing.
- **Tissue**
 - ① Homogenize in cold PBS (pH 7.0) on ice.
 - ② Centrifuge (4°C , 10,000 g, 10 min) and use the supernatant only.
- **Cell**
 - ① Perfusion 2-3 washes with cold PBS (pH 7.0).
 - ② Homogenize or sonicate in cold PBS (pH 7.0) on ice.
 - ③ Centrifuge (4°C , 10,000 g, 5 min) and use the supernatant only.

3. Reaction mixture preparation

1. Prepare 300 µL per tube by mixing the following in 1.5 mL microcentrifuge tubes.

Tube	Standard	Sample	Blank
Standard/Sample	standard 100 µℓ	sample 100 µℓ	sample 100 µℓ
Copper Reagent	100 µℓ	100 µℓ	100 µℓ
Reaction buffer	100 µℓ	100 µℓ	-
Ethanol	-	-	100 µℓ
Total volume	300 µℓ	300 µℓ	300 µℓ

2. Incubate at room temperature for 30 minutes.
3. Transfer 120 µL to each well of a 96-well plate and measure absorbance at 450 nm using a plate reader.

▪ Calculation

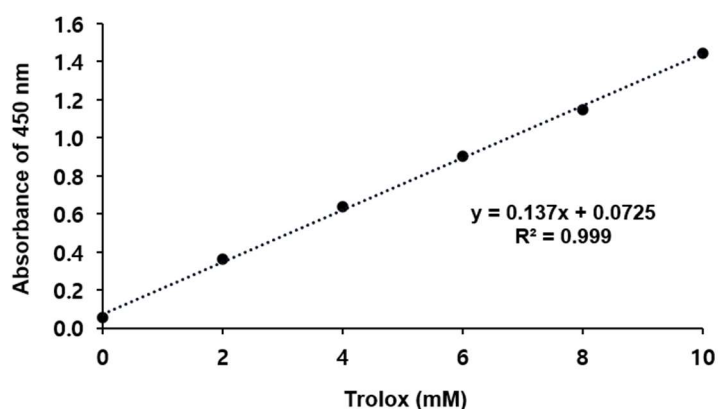
1. Subtract the O.D. value of the blank from all measurement values.
2. Plot the O.D. values (Y-axis) against the Trolox concentrations (X-axis) to generate a standard curve and determine the linear equation.
3. Calculate the concentration for each sample using the linear equation represented by the standard curve.

The concentration of Total Antioxidant Capacity in the sample. (C) = B/V x D (mM)

B : The amount of Trolox (in mM) in the measured well, determined from the standard curve.

V : The volume of the sample added into the well (in µℓ).

D : The dilution factor of the sample (e.g., for a 2x dilution, calculate as x2, not x1/2).



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-ABTS Antioxidant Assay Kit (Colorimetric)	DG-ABT400	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/VLDL 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