EZ-Ascorbic Acid Assay Kit

Metabolism assay kit

(Colorimetric)

Cat. No. DG-ASC100

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

▪ Product Description

Ascorbic Acid (Vitamin C) plays a vital role in numerous biological processes. It functions as a potent antioxidant, anti-inflammatory, antiviral agent, and immune stimulant, and is found in a variety of biological substances. Biological samples such as serum contain various antioxidants, most of which strongly react with Ascorbic Acid during analysis.

The EZ-Ascorbic Acid (FRASC) Assay Kit provides a fast, simple, and sensitive method for measuring Ascorbic Acid in biological samples such as serum, other bodily fluids, tissue and cell extracts, culture media, and food. In this assay, Fe^{3+} is reduced to Fe^{2+} by antioxidants, forming a product that strongly absorbs color in the range of 545-600 nm when chelated with the colorimetric probe.

▪ Contents and Storage Conditions

Component	100 assay	Cap Cord	Storage
Ascorbic Acid Assay buffer	25 ㎖	-	
Ascorbic Acid Enzyme	2 vials	Red	
Catalyst	1 ㎖	Blue	-20℃
Ascorbic Acid Probe	1 ㎖	Yellow	
Ascorbic Acid Standard (10mM)	1 vial	Green	

* This product is for research purposes only and should not be used for human consumption or diagnostic purposes.

▪ Preparation of Reagent

Component	Preparation	Storage and Stability
Ascorbic Acid Buffer	-	Store at room temperature for sufficient time before use.
Ascorbic Acid Enzyme	Add 250 ㎕ of D.W. and mix gently by inverting. Prepare immediately before use.	After use, store at -20°C and use within 2 weeks.
Ascorbic Acid Standard (10 mM)	Add 1 ㎖ of D.W. and mix gently by inverting. Prepare immediately before use.	Use the Ascorbic acid solution within 24 hours after adding distilled water.

* This protocol is optimized for use with a 96-well plate for experiments/measurements.
* Ensure that the enzyme and Ascorbic Acid Standard are completely dissolved before use.

▪ General Protocol

Sample preparation

Note

- If the sample volume is less than 100 ㎕, adjust the volume with distilled water (D.W.).
- Prepare samples at various concentrations to ensure that the O.D. values during absorbance measurement fall within the range of 0 to 2.5.
- Using hemolyzed serum or plasma may negatively affect the results.
- Heparin-treated plasma is recommended over EDTA plasma, as it is more stable.

1. Serum

① Collect blood in a tube without anticoagulant.

② Allow the blood to clot at room temperature for 30 minutes, then centrifuge at 2500 × g for 20 minutes.

③ Remove the supernatant and either use the sample immediately or store it frozen at -80°C.

2. Plasma

① Collect the sample and mix it in a tube containing heparin as an anticoagulant.

② Centrifuge at 3,000 rpm for 10–15 minutes at 4℃.

③ Remove the supernatant and either use the sample immediately or store it frozen at -80℃.

3. Cells and tissues

① Add D.W. to the cells and tissues, then perform sonication or homogenization on ice.

② Centrifuge at 3,000 rpm for 15 minutes at 4℃ and use the supernatant.

③ For more accurate measurements, prepare samples at various concentrations using the Ascorbic Acid Assay Buffer.

Ascorbic Acid Standard preparation

: To prepare a 1 mM Ascorbic Acid solution, mix 100 ㎕ of a 10 mM Ascorbic Acid solution with 900 ㎕ of distilled water.

Then, add distilled water to 0, 10, 20, 30, 40, and 50 ㎕ of the 1 mM Ascorbic Acid solution to adjust the final volume to 500 µL for each sample.

Standard No.	1 mM Ascorbic Acid Solution	D.W	Final standard volume in	Final Ascorbic Acid Conc. (nmol/well)
1	0 ㎕	500 ㎕	500 ㎕	0
2	10 ㎕	490 ㎕	500 ㎕	2
3	20 ㎕	480 ㎕	500 ㎕	4
4	30 ㎕	470 ㎕	500 ㎕	6
5	40 ㎕	460 ㎕	500 ㎕	8
6	50 ㎕	450 ㎕	500 ㎕	10

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

* For both the standard and sample, prepare at least 4 wells for each concentration. Two wells should be used without the enzyme, and the remaining 2 wells should be treated with the enzyme to be used as blanks.

Reaction mixture preparation

: This is the volume for one assay. Calculate the amount of assay needed for your experiment and prepare the reaction mixture with the calculated assay amount plus 1.

Components	Reaction mixture
Ascorbic Acid Buffer	80 ㎕
Ascorbic Acid Probe	10 ㎕
Catalyst	10 ㎕
Total	100 ㎕

Ascorbic Acid (FRASC) Assay

	Ascorbic Acid	Ascorbic Acid Blank	Sample	Sample Blank
Ascorbic Acid standard solution	100 ㎕	100 ㎕	-	-
Sample	-	-	100 ㎕	100 ㎕
Ascorbic Acid enzyme	-	10 ㎕	-	10 ㎕
D.W	10 ㎕	-	10 ㎕	-
Incubate the reaction at room temperature for 15 minutes.				
Reaction mixture	100 ㎕	100 ㎕	100 ㎕	100 ㎕
After dispensing the reaction mixture, measure the absorbance at 593 nm within 2 to 3 minutes.				

* The Blank is used to selectively remove only the Ascorbic Acid by the enzyme and then measure the absorbance.

① Before the experiment, store the Buffer, Catalyst, and Probe at room temperature and ensure they are fully dissolved before use.

② Prepare 100 ㎕ of standard and sample in each concentration, with a minimum of 4 wells per concentration on the 96-well plate.

③ For each concentration, add 10 ㎕ of D.W. to 2 wells, and add 10 ㎕ of Ascorbic Acid Enzyme to the other 2 wells for the blank. Mix thoroughly for about 30 seconds using a plate shaker.

* If mixing is insufficient, accurate measurements may not be possible. If no plate shaker is available, mix thoroughly with a pipette.

④ Incubate at room temperature for 15 minutes.

⑤ Add 100 ㎕of the pre-prepared reaction mix to all wells, and measure the absorbance at 593 nm within 2 to 3 minutes.

▪ Calculation

1. Correct the background O.D. values in all measurements.
   * Background = $O.D_{593nm}$ from Ascorbic Acid standard No.1 (0 μM Ascorbic Acid)

2. Calculate the average of duplicate measurements for each standard well and sample well.

3. Correct each concentration's Ascorbic Acid and sample average values by subtracting the blank average value.

4. Plot a standard curve using the Ascorbic Acid standard O.D. values
5. Substitute the sample O.D. values into the standard curve to calculate the amount of Ascorbic Acid in the sample.
6. Based on the Ascorbic Acid amount calculated in step 5, use the following formula to calculate the concentration of Ascorbic Acid in the sample.

Concentration of Ascorbic Acid in the sample (C)
= B/V x D (nmol/㎕ or μmol/ml or mM)

B: Amount of Ascorbic Acid in the measurement well obtained from the standard curve (nmol)
V: Amount of sample in a well (100 ㎕)
D: Sample dilution ratio (e.g., 2-fold dilution, multiply by 2).

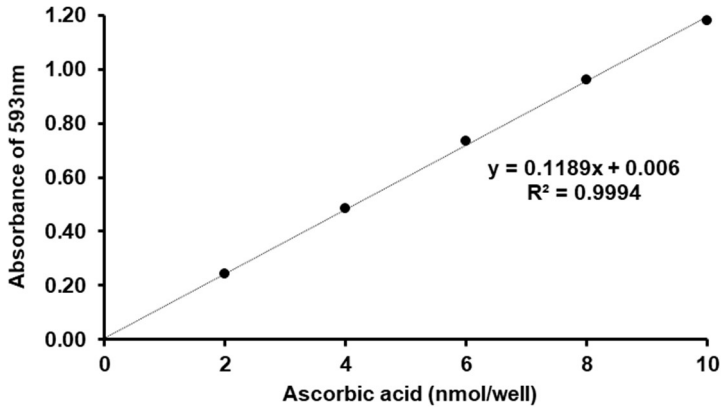

Fig. Ascorbic acid standard curve. Assay was performed following the kit protocol.

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▪ 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
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