Instruction manual

* For research use only
* Store at 4 – 8℃

Total Antioxidant Capacity Assay kit
(Copper Reduction Ability assay method)

Description

Reactive Oxygen Species (ROS) can cause damage to biomolecules such as DNA, proteins, lipids etc. To protect from such damages, organisms produce and ingest various antioxidants that interact with and neutralize ROS. An imbalance between the production and scavenging of ROS is the underlying basis of oxidative stress, that is thought to be involved in the development of cancer, Parkinson's disease, Alzheimer's disease, atherosclerosis, heart failure etc.

Measurements of Total Antioxidant Capacity (TAC) were used as an integrated index rather than the simple sum of measurable antioxidants. It has been reported that patients of various disease and aged people exhibit low serum TAC levels. Therefore, TAC is greatly expected as a marker of aging and diseases.

Total Antioxidant Capacity Assay kit measures not only the TAC of biological fluid such as plasma, serum and urine etc. but also that of foods such as wine, beverage, tea etc.

Antioxidants in the sample reduce Cu^{2+} to Cu^{+}. Cu^{+} is chelated by bathocuproine and yields an orange colored complex. Since the intensity of the color formed is proportional to the TAC, TAC can be determined by measuring absorbance of the generated complex at wavelength 490 nm (430 nm to 510 nm).

Kit contents

200 tests （Catalog # : AC01DE ）

R-A	Buffer solution (bathocuproine)	30 mL×1
R-B	Cupric ion (Cu^{2+}) solution	14 mL×1
R-C	Stop solution	6 mL×1
Standard (STD)	Calibrator (Ascorbic acid 1.0mM)	Vial×1

Note

A) Reaction time has a significant impact on the accuracy and reproducibility of the measured values. Control the reaction time exactly. We recommend the use of multi-channel pipette.

B) Use disposable test tube and glassware washed with 1 M HNO_3 or 1 M HCl solution and distilled water.

C) In the cell lysate or the tissue extract use as specimen, high concentration of proteins or lipid, may affect observed value. Please remove them by ultrafiltration or centrifugation.

D) Accuracy in pipetting volume for samples and reagents may affect the quality of assay. Please note that samples, standards and Working Reagent must be poured accurately µL level.

E) Temperature for chromogen reaction may affect optical density. Please try to extend or shorten chromogen reaction time depending on room temperature.

F) Sample must be assayed fresh. If you stored the samples, please keep in -20 ℃.

G) Repeated freeze/thawing causes damage to sample.

H) Sample, which containing high concentration copper or copper chelating agent, may not be measured accurately.

Required but not provided:

- Micro plate reader (Wavelength 430-510 nm)
- 96 well
- Pipette (5 µL)
- Multi-cannel pipette (30µL, 70µL, 150µL) and reservoir.
- Distilled water

Operation

1. Preparation of samples

◇Serum or Plasma

Pre-dilution is not required.

Do not use a blood sampling tube with EDTA.

Hemolized sample cannot be used.

◇Cell lysate

Insoluble solid in lysate should be removed by filtration or centrifugation.

◇Biomedical tissue

When you measure the TAC of water-soluble antioxidants in a biomedical tissue, extract it by the following method.

＜Extracting method＞

1.) Wash away blood from tissue with saline.
2.) Mince or homogenize the tissue.
3.) Add 10 mL of distilled water or lysis buffer to 10 g of the tissue.
4.) Centrifuge the mixture (10,000 rpm, 10 min, 4℃).
5.) Collect the supernatant and use it as a sample.

◇Other samples(such as beverages)

It is necessary to dilute samples when the antioxidant concentration (ascorbic acid equivalent) of the samples is over 3 mM. See the below table, and dilute samples with distilled water or saline.

Ex:

Sample	Dilution rate (-)	Ascorbic acid equivalent (mM)
Serum or plasma	Not required	0.42
Black tea	×8	7.59
Green tea	×8	3.25
Coffee	×25	5.93
Red wine	×8	15.8
White wine	×4	1.37
Rice wine	Not required	0.51
Fruits juice	×4	1.43

2. Assay preparation

- R-A, R-B, R-C
  Bring all reagents to under room temperature before use.

- Reconstitute of standards
  Add distilled water to STD vial to dissolve ascorbic acid. (The volume of distilled water to add is indicated on the label of the vial.)

3. Assay procedure

Quantitative analysis with microplate reader.

(255 µL per 1 assay sample)

○Assay

(1) Add 5 µL of distilled water (Blank) / STD / sample into each well.
(2) Add 150 µL of R-A to each well and incubate at room temperature for 5 min.
(3) Read the absorbance at 490 nm. →OD1
(4) Add 70 µL of R-B to each well and incubate at room temperature for 5 min.
(5) Add 30 µL of R-C to each well.
(6) Read the absorbance at 490 nm. →OD2
* Select the filter: 430 - 510 nm at 490 nm (main).

		Assay Sample		
Add	(µL)	Blank OD_{Bl}	Standard OD_{Std}	Sample OD_S
1	Distilled water	5		-
	STD	-	5	-
	Assay sample	-	-	5
2	R-A	150	150	150
	↓ Mix and incubate for 5 min at room temperature. Read the absorbance at 490 nm. (OD1)			
3	R-B	70	70	70
	↓ Mix and incubate for 5 min at room temperature.			
4	R-C	30	30	30
	↓ Mix and read the absorbance at 490 nm. (OD2)			

○Calculations

$\Delta OD_{Std} = (OD2_{Std} - OD1_{Std}) - (OD2_{Bl} - OD1_{Bl})$

$\Delta OD_S = (OD2_S - OD1_S) - (OD2_{Bl} - OD1_{Bl})$

Total antioxidant capacity (mM)= $\Delta OD_S/\Delta OD_{Std}$ X 1.0
(Ascorbic acid equivalent)

Copper reduction ability (mM) = $\Delta OD_S/\Delta OD_{Std}$ X 2.0

* When a sample was diluted, please multiply the result by dilution-factor.

(Assay example)

	OD1 (490nm)	OD2 (490nm)	OD	ΔOD	Ascorbic acid equivalent (mM)
Blank	0.029	0.041	0.012	-	-
Standard	0.031	0.302	0.271	0.259	-
Sample	0.070	0.174	0.104	0.092	0.355

Performance

Measuring range 0.05 – 3.0 mM (Ascorbic acid equivalent)

Imprecision Imprecision was evaluated using serum.

Within run

	Mean mM	S.D	C.V %
Level 1	0.33	0.01	6.10
Level 2	0.74	0.02	5.72

Run to run

	Mean mM	S.D	C.V %
Level 1	0.33	0.02	3.47
Level 2	0.74	0.03	2.71

Expiration date and preservation conditions

Storage conditions: Store at 2-8°C. Don't freeze.
Expiration: Use before 12 months from date of manufacture.
After the bottles are opened, the kit should be used in 1 month.

Reference

1.) Campos C, Guzmán R, López-Fernández E, Casado A. Anal Biochem. "Evaluation of the copper(II) reduction assay using bathocuproinedisulfonic acid disodium salt for the total antioxidant capacity assessment: the CUPRAC-BCS assay." *2009 Sep 1;392(1):37-44. doi: 10.1016/j.ab.2009.05.024. Epub 2009 May 21.*

2.) Yuji Naito, Masaichi-Chang-il Lee, Yoji Kato, Ryoji Nagano, Yoshikazu Yonei "Oxidative Stress Markers." *Anti-Aging Medicine 7(5):36-44,2010*

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