Total Antioxidant Status (TAS) Colorimetric Assay Kit

Catalog Number EEA025 (96 tests)

Rev 2.0

For safety and biohazard guidelines, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The kit is used for the determination of total antioxidant status (TAS) in serum, plasma, urine, cellular supernatant, animal and plant tissue samples. ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) can be oxidized to $ABTS^{\bullet+}$ (a green-blue color in solution), which can be further reduced back to the colorless ABTS in the presence of antioxidants. The total antioxidant status (TAS) of the sample can be determined and calculated by measuring the absorbance of $ABTS^{\bullet+}$ at 660 nm. Trolox is an analog of Vitamin E with a similar antioxidant state. As such, Trolox is used as a reference substance for total antioxidant status.

Contents and storage

Kit and components are shipped at -20 ℃. An unopened kit can be stored at -20 ℃ for 12 months.

Components	Quantity (96 tests)
Buffer Solution	30 mL
Chromogenic Agent	5 mL
2 mmol/L Standard	4 mL
Microplate	1 plate
Plate Sealer	2 pieces

Required materials

- Distilled or deionized water
- 60% Ethanol, PBS (0.01 M, pH 7.4)
- Microtiter plate reader with software capable of measurement at or near 660 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions.
- Incubator capable of maintaining 37 ℃.

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Sample preparation guidelines

Sample requirements

- The samples should not contain SDS, Tween20, NP-40, Triton X-100 and other detergents, and should not contain DTT, 2-merhydryl ethanol, or other reducing reagents.

Serum and plasma samples: Detect the sample directly. If the sample is turbid, centrifuge at 10000 g for 10 min at 4 ℃, then take the supernatant for detection.

Tissue sample:

- Take 0.02-1 g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8 ℃ to remove blood cells.
- Absorb the water with filter paper and weigh.
- Homogenize at the ratio of the volume of 60% ethanol (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4 ℃.
- Take the supernatant and preserve it on ice for detection.

If not detected on the same day, the tissue sample (without homogenization) can be stored at -80 ℃ for 1 month.

Prepare samples

It is recommended to take 2~3 samples with expected large difference to do a pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.23-2 mmol Trolox Equiv. /L)).

The recommended dilution factor for different samples is as follows (for reference only):

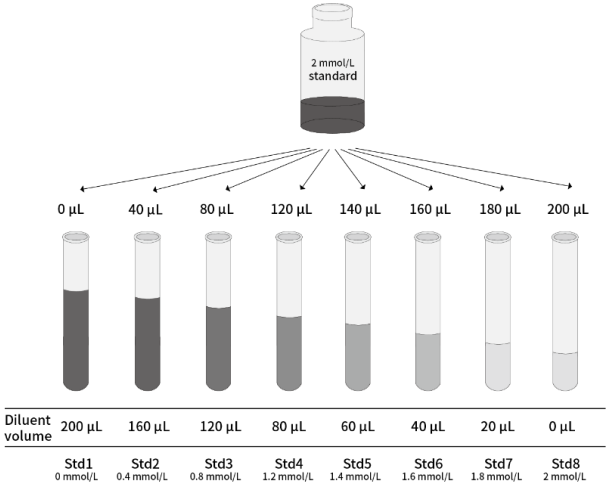
Sample type	Dilution factor
10% Mouse liver tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Rat lung tissue homogenate	1
Molt4 cellar supernatant	1
Human urine	8-10
Mouse serum	1
Human serum	1
Human saliva	1

Note: The diluent is 60% ethanol.

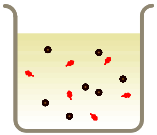
Prepare diluted standards

Note: Use glass or plastic tubes for diluting standards.

Dilute 2 mmol/L standard solution with 60% ethanol to a serial concentration. The recommended dilution gradient is as follows: 0, 0.4, 0.8, 1.2, 1.4, 1.6, 1.8, 2 mmol/L.



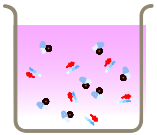
Assay Protocol



1. Add sample and standard

a. **Sample well:** Add 10 μL of sample to the sample well.

b. **Standard well:** Add 10 μL of standard with different concentrations to the standard wells.



2. Add substrate

a. Add 200 μL of buffer solution to each well.

b. Measure the OD values of each well at 660 nm with microplate reader, recorded as A_1.

c. Add 20 μL of chromogenic agent to each well, pipetting up and down 5-6 times until fully mixed.

d. Incubate at 37 ℃ for 5 min. Measure the OD values of each well at 660 nm with microplate reader, recorded as A_2. $\Delta A=A_2-A_1$.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean ΔA value of all standard from the blank (Std1). This is the absoluted ΔA value.
3. Plot the standard curve by using absoluted ΔA value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

Liquid sample (Trolox is used as a reference substance for total antioxidant status):

$$\text{TAS (mmol Trolox Equiv. /L)} = (\Delta A_{Blank} - \Delta A_{Sample} - b) \div a \times f$$

Tissue sample:

$$\text{TAS (mmol Trolox Equiv. /kg wet weight)} = (\Delta A_{Blank} - \Delta A_{Sample} - b) \div a \div (m \div v) \times f$$

[Note]

y: $\Delta A_{Blank} - \Delta A_{Standard}$ (ΔA_{Blank} is ΔA when the standard concentration is 0);
x: The concentration of standard;
a: The slope of standard curve;
b: The intercept of standard curve.
ΔA_{Sample}: The OD value of sample (A_2-A_1).
m：The weight of tissue sample (g);
f: Dilution factor of sample before test.
V：The volume of added homogenate (mL)

To easy calculate the test results, refer to the calculation file available on the webpage.

Example analysis

For human serum, take human serum sample and carry the assay according to the operation steps. The results are as follows:

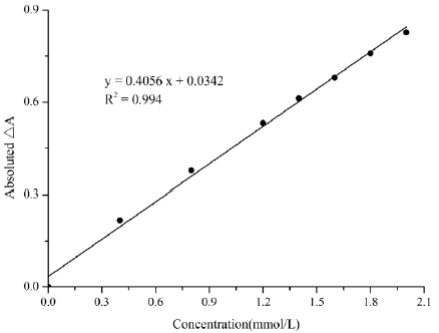
standard curve: y = 0.4056 x + 0.0342, the OD value of the sample (A_1) is 0.08, the OD value of the sample (A_2) is 0.777, $\Delta A_{Sample} = A_2 - A_1 = 0.697$, ΔA_{Blank} is 1.196, and the calculation result is:

TAS (mmol Trolox Equiv./L) = (1.196–0.697 -0.0342) ÷ 0.4056 =1.14 mmol Trolox Equiv. /L

Performance characteristics

- Standard curve (example)

The following data were obtained for the various standards over the range of 0–2 mmol/L standard.

Concentration (mmol/L)	ΔA at 660 nm	Standard Curve
2.00	0.368	
1.80	0.436	
1.60	0.516	
1.40	0.583	
1.20	0.664	
0.80	0.818	
0.40	0.978	
0.00	1.196	

- Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol Trolox Equiv./L)	0.87	1.05	1.46
%CV	4.8	4.5	4.5

CV = Coefficient of Variation

- Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol Trolox Equiv./L)	0.87	1.05	1.46
%CV	6.8	7.3	6.9

CV = Coefficient of Variation

- Expected values

This assay was tested with human serum samples without dilutions.

Sample Type	Range (**mmol Trolox Equiv. /L**)	Average (**mmol Trolox Equiv. /L**)
Human serum	1.20-1.50	1.35

- Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 96.7%.

	Sample 1 (low conc.)	Sample 2 (middle conc.)	Sample 3 (high conc.)
Expected Conc. (mmol/L)	0.62	0.98	1.85
Observed Conc. (mmol/L)	0.6	0.96	1.79
Recovery rate (%)	95.6	98	96.6

- Recommended Plate Set Up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80
[Note]: A-H, standard wells; S1-S80, sample wells												

- Sensitivity

The analytical sensitivity of the assay is 0.23 mmol Trolox Equiv. /L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times and calculating the corresponding concentration.

Limited product warranty

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