TECHNICAL MANUAL

Total Antioxidant Status (TAS) Colorimetric Assay Kit

- **SKU CODE:** MAES0198
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Incubate and measure absorbance

2. Intended use

This kit is used for the determination of total antioxidant status (TAS) in serum, plasma, urine, cellular supernatant, and animal and plant tissue samples.

3. Detection principle

ABTS is oxidized to green ABTS+ by an appropriate oxidant, which can be reduced to colorless ABTS in the presence of antioxidants. The TAS of the sample can be determined and calculated by measuring the absorbance of ABTS+ at 660 nm. Trolox is an analog of vitamin E and has a similar antioxidant state to that of vitamin E. Trolox is used as a reference substance for total antioxidant status.

4. Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500 Assays)	Storage
Reagent 1	Buffer Solution	15 mL x 1 vial	30 mL x 1 vial	50 mL x 3 vials	-20 °C, 12 months
Reagent 2	Chromogenic Agent	5 mL x 1 vial	5 mL x 1 vial	25 mL x 1 vial	-20 °C, 12 months, protect from light
Reagent 3	2 mmol/L Standard	2 mL x 1 vial	4 mL x 1 vial	20 mL x 1 vial	-20 °C, 12 months, protect from light
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces	2 pieces		

5. Materials prepared by users

Instruments:

Microplate reader (650-670 nm, optimum wavelength: 660 nm), micropipettes, 37 °C incubator

Reagents:

Double distilled water, 60% ethanol

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 2 mmol/L standard solution with double distilled water 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.

7. Standard dilution table

Item	Concentration (mmol/L)	2 mmol/L Standard (μL)	Double distilled water (μL)
①	0	0	200
②	0.4	40	160
③	0.8	80	120
④	1.2	120	80
⑤	1.4	140	60
⑥	1.6	160	40
⑦	1.8	180	20
⑧	2	200	0

8. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80 °C for one month.

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

2. Wash tissue in cold PBS (0.01 M, pH 7.4).

3. Homogenize 20 mg tissue in 180 µL 60% ethanol with a dounce homogenizer at 4 °C.

4. Centrifuge at 10,000 xg for 10 minutes at 4 °C to remove insoluble material. Collect supernatant and keep it on ice for detection.

Dilution of sample:

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 cellular supernatant - 1

Human urine - 8-10

Mouse serum - 1

Human serum - 1

Human saliva - 1

Note: The diluent is 60% ethanol. For the dilution of other sample types, please perform a pre-test to confirm the dilution factor.

9. The key points of the assay

1. After adding the chromogenic reagent, mix the solution thoroughly by pipetting up and down several times.
2. When adding samples, avoid introducing bubbles.

10. Operating steps

1. Standard well: Add 10 µL of standard with different concentrations to the standard well. Sample well: Add 10 µL of sample to the sample well.
2. Add 200 µL of buffer solution to each well.

3. Measure the OD values of each well at 660 nm with microplate reader, recorded as A1.
4. Add 20 μL of chromogenic agent to each well, repeatedly suck and beat for 5-6 times.
5. Incubate at 37 °C for 5 minutes. Measure the OD values of each well at 660 nm with microplate reader, recorded as A2. ΔA = A2 - A1.

11. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean ΔA value of all standards from the blank (Standard #①). This is the absolute ΔA value.

3. Plot the standard curve by using absolute 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).

The sample:

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

TAS (mmol Trolox Equiv./L) = (ΔA_Blank – ΔA_Sample - b) ÷ a × f

2. Tissue sample:

TAS (mmol Trolox Equiv./kg wet weight) = (ΔA_Blank – ΔA_Sample - b) ÷ a ÷ (m ÷ v) × f

[Note]

y: ΔA_Blank – ΔA_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).

v: The volume of added homogenate (mL).

f: Dilution factor of sample before test.

12. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Intra-assay Precision Table

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

Inter-assay Precision

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

Inter-assay Precision Table

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

Recovery

Three samples of high, middle, and low concentration were tested with each concentration run 6 times in parallel to obtain an average recovery rate of 99%.

Recovery Table

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.7	1.35	1.75
Observed Conc. (mmol/L)	0.7	1.3	1.7

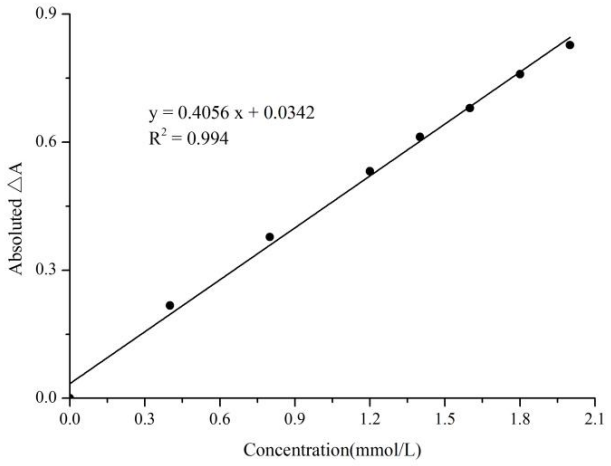
	Standard 1	Standard 2	Standard 3
Recovery rate (%)	101	99	97

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.

Standard curve

As the ΔA value of the standard curve may vary according to the conditions of the actual assay performance (e.g., operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only:



Standard curve data

Concentration (mmol/L)	Average ΔA	Absolute ΔA
0	1.196	0.000
0.4	0.978	0.217
0.8	0.818	0.378
1.2	0.664	0.532
1.4	0.583	0.612

Concentration (mmol/L)	Average ΔA	Absolute ΔA
1.6	0.516	0.680
1.8	0.436	0.759
2	0.368	0.827

13. Appendix II Example Analysis

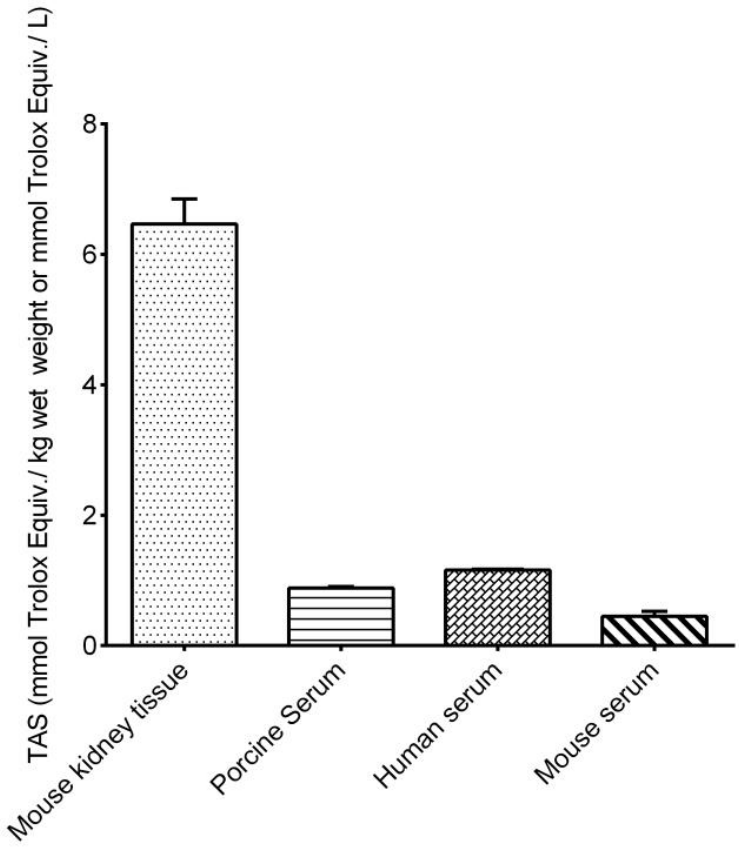
Example analysis:

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

Standard curve: y = 0.4056x + 0.0342, the OD value of the sample (A_1) is 0.08, the OD value of the sample (A_2) is 0.777, ΔA_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

Detect 10% mouse kidney tissue homogenate (diluted 4 times), human serum, mouse serum and porcine serum according to the protocol. The results are as follows:



14. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use

of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

15. Appendix III Products Cited In Published Literatures

1. Guler A, Yilmaz A, Oncer N, et al. Machine learning-assisted SERS approach enables the biochemical discrimination in Bcl-2 and Mcl-1 expressing yeast cells treated with ketoconazole and fluconazole antifungals. Talanta, 2024, 276. IF: 6.1
2. Oi-Jurjevi B, Borkovi-Miti S, Pavlovi S, et al. Lemon Flavonoid Extract Eriomin Improves Pro/Antioxidant Status and Interferes with Cholesterol Metabolism without Affecting Serum Cholesterol Levels in Aged Rats. International Journal of Molecular Sciences, 2024, 25(10).
3. Prvulovic M, Pavlovic S, Mitic S B, et al. Mitigating the effects of time in the heart and liver: The variable effects of short- and long-term caloric restriction. Mechanisms of Ageing and Development, 2024, 222.
4. Kara M, Sahin S, Rabbani F, et al. An in vitro analysis of an innovative standardized phospholipid carrier-based Melissa officinalis L. extract as a potential neuromodulator for emotional distress and related conditions. Frontiers in Molecular Biosciences, 2024. DOI:10.3389/fmolb.2024.1359177.
5. Khan A. In Vitro Mechanistic Studies of a Standardized Sustainable Grape Seed Extract for Potential Application as a Mood-Modulating and Cognition-Enhancing Supplement. Nutrients, 2024, 16. DOI:10.3390/nu16203459.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.