TECHNICAL MANUAL

Total Antioxidant Capacity (T-AOC) Colorimetric Assay Kit (FRAP)

- **SKU CODE:** MAES0150
- **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 at 37°C
- Measure absorbance at 593 nm

2. Intended use

This kit can be used to measure total antioxidant capacity (T-AOC) in serum, plasma, tissue homogenate, cell, cell culture supernatant, saliva and urine samples.

3. Detection principle

Fe3+-TPTZ can be reduced by antioxidants and produce blue Fe2+-TPTZ under acidic conditions. The antioxidant capacity of the sample can be calculated by detecting the absorbance value at 593 nm.

4. Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500 Assays)	Storage
Reagent 1	Buffer Solution	10 mL × 1 vial	20 mL × 1 vial	50 mL × 2 vials	2-8°C, 12 months
Reagent 2	TPTZ Solution	1 mL × 1 vial	2 mL × 1 vial	10 mL × 1 vial	2-8°C, 12 months, protected from light
Reagent 3	Substrate Solution	1 mL × 1 vial	2 mL × 1 vial	10 mL × 1 vial	2-8°C, 12 months, protected from light

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500 Assays)	Storage
Reagent 4	FeSO4•7H2O Standard	100 mg × 1 vial	200 mg × 1 vial	200 mg × 5 vials	2-8°C, 12 months
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (590-600 nm), Electronic balance, 37°C Constant temperature incubator, Micropipettor

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

6. Reagent preparation

1. **Preparation of FRAP working solution:** For each well, prepare 180 µL of FRAP working solution (mix well 150 µL of buffer solution, 15 µL of TPTZ solution and 15 µL of substrate solution). Use the FRAP working solution within 2 hours. The FRAP working solution should be prepared fresh.
2. **Preparation of 100 mM FeSO4 solution:** Dilute 27.8 mg of FeSO4·7H2O standard accurately with 1 mL of double distilled water. The 100 mM FeSO4 solution should be prepared fresh. Note: Fe2+ is easily oxidized to Fe3+, the color will change from light green to light yellow. Please discard the solution if its color is yellow.
3. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 100 mmol/L FeSO4 solution with double distilled water to 10 mmol/L, and then dilute with distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.3, 0.6, 0.9, 1.2, 1.8, 2.1, 2.5 mmol/L.

7. 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.

Urine: Collect fresh urine and centrifuge at 10,000 xg for 10 min at 4°C. Take the supernatant and preserve it on ice for detection. If not detected on the same day, the urine can be stored at -80°C for one month.

Saliva: Gargle with clear water, collect the saliva 30 min later, centrifuge at 10,000 xg for 10 min at 4°C. Take the supernatant and preserve it on ice for detection.

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 PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4°C.

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

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Cells:

1. Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

2. Wash cells with PBS (0.01 M, pH 7.4).

3. Homogenize 1×10^6 cells in 300-500 µL PBS (0.01 M, pH 7.4) with an ultrasonic cell disruptor at 4°C.

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

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

The recommended dilution factor for different samples is as follows (for reference only): Human serum (1), Human saliva (1), Human urine (1), Cellular supernatant (1), HepG2 cells homogenization (1), 5% Mouse liver tissue homogenization (1), 10% Epipremnum aureum tissue homogenization (1).

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. Reagents that are blue or approximately blue in acidic conditions will interfere with the detection results and should be avoided if possible.
2. High concentrations of Fe3+ salt or Fe2+ salt in samples may interfere with the results, as they will inhibit the interference of endogenous substances in samples under acidic conditions. The total concentration of Fe3+ salt or Fe2+ in serum (plasma) is usually lower than 10 μM, which will not interfere with FRAP detection. Small amounts of metal chelating agents in samples will not affect the detection.
3. Substances that may affect oxidation-reduction reactions (e.g., DTT and mercaptoethanol) and detergents (e.g., Tween, Triton and NP-40) cannot be added to samples.
4. It is recommended to store samples at -80°C if detection cannot be performed immediately. The detection results will not change significantly within 1 month.
5. TPTZ solution is irritating to humans; please wear laboratory coat and gloves during operation.

9. Operating steps

1. **Standard well: Add 5 μL of standard with different concentrations into the standard wells.:** Sample well: Add 5 μL of sample into the sample wells.
2. Add 180 μL of FRAP working solution to each well.
3. Incubate at 37°C for 3-5 min, then measure the OD values of each well with microplate reader at 593 nm.

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This is the absolute OD value.

3. Plot the standard curve by using absolute OD value of standard and corresponding concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

The sample:

1. Serum (plasma) and other liquid samples:

T-AOC (mmol/L) = $(A_{593} - b) \div a \times f$

2. Tissue and cell samples:

T-AOC (mmol/gprot) = $(A_{593} - b) \div a \div C_{pr} \times f$

[Note]

A_{593}: $OD_{Sample} - OD_{Blank}$.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, gprot/L.

11. 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

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.58	1.02	1.95
%CV	4.3	4.0	3.4

Inter-assay Precision

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

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.58	1.02	1.95
%CV	7.6	8.4	8.3

Recovery

Three samples of high, medium, and low concentrations were tested with each concentration performed 6 times in parallel to obtain an average recovery rate of 103%.

Recovery

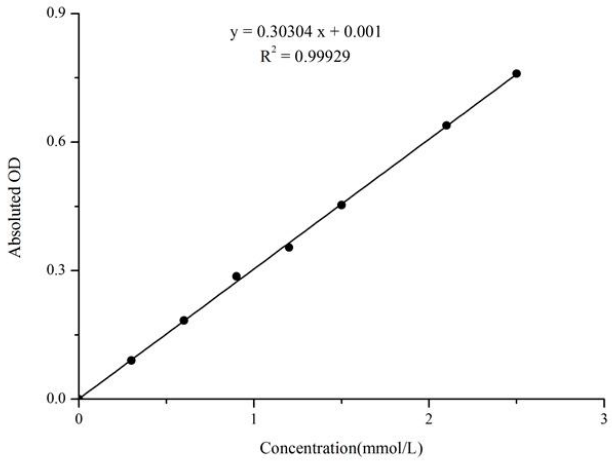
Standard 1	Standard 2	Standard 3
0.5	1.1	2
0.5	1.2	2.1
101	105	103

Sensitivity

The analytical sensitivity of the assay is 0.049 mmol/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 OD 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

Concentration (mmol/L)	0	0.3	0.6	0.9	1.2	1.8	2.1
Average OD	0.070	0.160	0.253	0.356	0.423	0.618	0.708

Concentration (mmol/L)	0	0.3	0.6	0.9	1.2	1.8	2.1
Absolute OD	0	0.090	0.184	0.287	0.354	0.548	0.639

12. Example Analysis

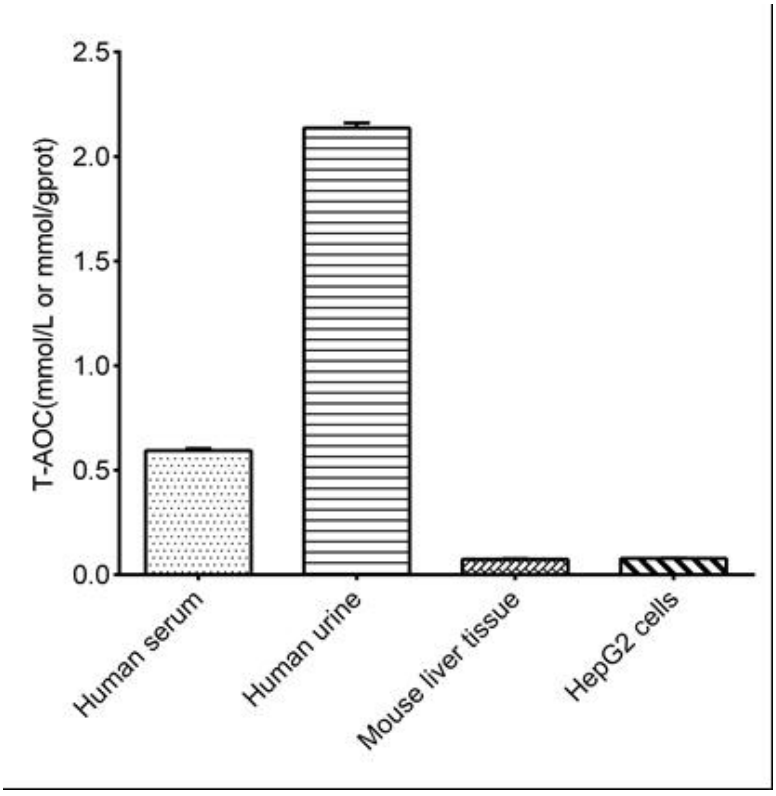
Example analysis:

Take 5 µL of human serum and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.30304x + 0.001, the average OD value of the sample is 0.2491, the average OD value of the blank is 0.0572, and the calculation result is:

T-AOC (mmol/L) = (0.2491 - 0.0572 - 0.001) ÷ 0.30304 × 1 = 0.63 (mmol/L)

Detect human serum, human urine, 5% mouse liver tissue homogenate (the concentration of protein is 2.30 gprot/L), HepG2 cells (the concentration of protein is 2.98 gprot/L) according to the protocol. The results are as follows:



13. 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.

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.