（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K225-M

Specification：48T(32 samples)/96T(80 samples)/96T×5(464 samples)

Measuring instrument：Microplate reader（590-600 nm）

Detection range：0.049-2.5 mmol/L

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

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service

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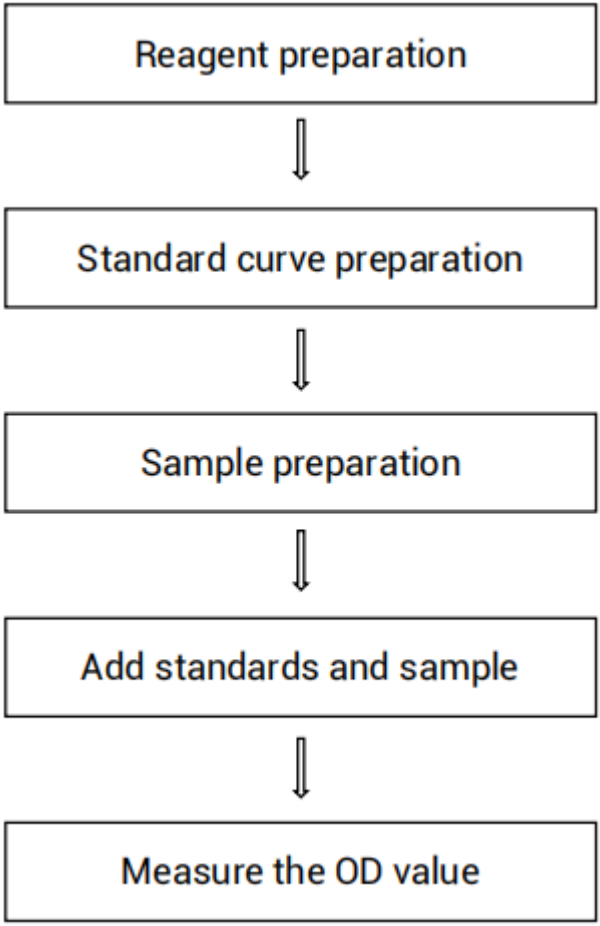
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Assay summary



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.

Detection principle

Fe^{3+}-TPTZ can be reduced by antioxidants and produce blue Fe^{2+}-TPTZ under acid condition. The antioxidant capacity of sample can be calculated by detection the absorbance value at 593 nm.

Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (96 T×5)	Storage
Reagent 1	Buffer Solution	10 mL×1 vial	20 mL×1 vial	50 mL × 2 vials	2-8℃, 12 months
Reagent 2	TPTZ Solution	1 mL×1 vial	2 mL×1 vial	10 mL × 1 vial	2-8℃, 12 months shading light
Reagent 3	Substrate Solution	1 mL×1 vial	2 mL×1 vial	10 mL × 1 vial	2-8℃, 12 months shading light
Reagent 4	$FeSO_4•7H_2O$ Standard	100 mg×1 vial	200 mg×1 vial	200 mg × 5 vials	2-8℃, 12 months
	Microplate	48 wells	96 wells	96 wells×5	No requirement
	Plate Sealer	2 pieces		7 pieces	
	Sample Layout Sheet	1 piece		7 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

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

Reagent preparation

① The preparation of FRAP working solution：
For each well, prepare 180 uL 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 on spot.

② The preparation of 100 mM $FeSO_4$ solution：
Dilute 27.8 mg of $FeSO_4 \cdot 7H_2O$ standard accurately with 1mL of double distilled water. The 100 mM $FeSO_4$ solution should be prepared on spot.
Note: Fe^{2+} is easily oxidized to Fe^{3+}, the color will change from light green to light yellow. Please discard the solution if it color is yellow.

③ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 100 mmol/L $FeSO_4$ solution with double distilled water to 10 mmol/L, and then diluted 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. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.3**	**0.6**	**0.9**	**1.2**	**1.8**	**2.1**	**2.5**
10 mmol/L $FeSO_4$ solution	0	15	30	45	60	90	105	125
Double distilled water (μL)	500	485	470	455	440	410	395	375

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Urine: Collect fresh urine and centrifuge at 10000 g for 10 min at 4℃. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the urine can be stored at-80℃ for a month.

Saliva: Gargle with clear water, collect the saliva 30 min later, centrifuge at 10000 g for 10 min at 4℃. 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℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

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 a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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

Sample type	Dilution factor
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 do pretest to confirm the dilution factor

The key points of the assay

① Reagents which are blue or approximate blue in acidic condition will make influence on the detection result should be avoided as possible.

② High concentration of Fe^{3+} salt or Fe^{2+} salt in samples may interfere the result, because they will inhibit the interference of endogenous substances in samples under acid condition. Total concentration of Fe^{3+} salt or Fe^{2+} in serum (plasma) is always lower than 10 μM, which will not interfere the FRAP detection. Small amount of metal chelating agent in samples will not affect the detection.

③ Substances which may affect the oxidation-reduction reaction (e.g., DTT and mercaptoethanol) and detergent (e.g., Tween, Triton and NP-40) cannot be added into samples.

④ It is recommended to store samples at -80ºC if the detection cannot be operated timely. The detection result will not change obviously within 1 month.

⑤ TPTZ solution is irritant for humans, please wear lab-gown and gloves during the operation.

Operating steps

① Standard well: Add 5 μL of standard with different concentration 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 ºC for 3~5 min, then measure the OD values of each well with Microplate reader at 593 nm.

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 absoluted OD value.

3. Plot the standard curve by using absoluted OD 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. Serum (plasma) and other liquid sample:

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

2. Tissue and cells sample:

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

[Note]

ΔA_{593}: $OD_{Sample} - OD_{Blank}$.

f: Dilution factor of sample before test.

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

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Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

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.

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

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 103%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.5	1.1	2
Observed Conc. (mmol/L)	0.5	1.2	2.1
Recovery rate (%)	101	105	103

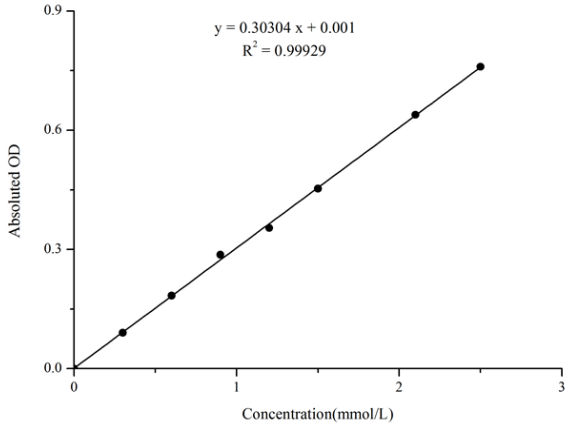
Sensitivity

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.

2. 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), so the standard curve and data are provided as below for reference only:

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
Absoluted OD	0	0.090	0.184	0.287	0.354	0.548	0.639



Appendix Π Example Analysis

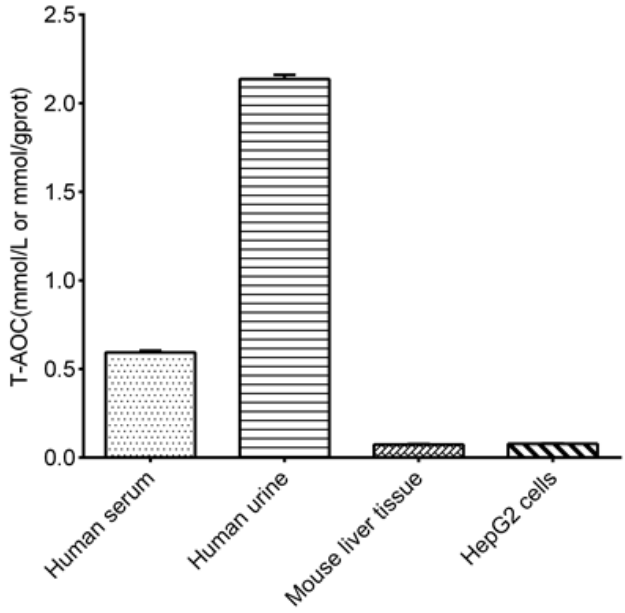
Example analysis：

Take 5 μL of human serum, carry 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 result is as follows：



Appendix III Publications

1. Liu Q , Wang L , Wang Z ,et al.Preparation and characterization of carvacrol/soybean protein isolate composite film with efficient antimicrobial and antioxidant activities and its application in grape preservation[J].Food Chemistry, 2025, 464.DOI:10.1016/j.foodchem.2024.141572.
2. Marhamati S , Younesian O , Mir S M ,et al.The effects of high doses of selenium supplementation on mRNA and protein levels of cMLCK levels and total antioxidant capacity in rat heart tissue[J].Food and Chemical Toxicology, 2024, 191(000):4.DOI:10.1016/j.fct.2024.114886.
3. Tefnu C L .The Interplay between Oxidative Stress and Fatty Acids Profile in Romanian Spotted Cows with Placental Retention[J].Veterinary Sciences, 2024, 11.DOI:10.3390/vetsci11100499.
4. Marchetti L , Rebucci R , Lanzoni D ,et al.Dietary supplementation with a blend composed of carvacrol, tannic acid derived from Castanea sativa and Glycyrrhiza glabra, and glycerides of medium chain fatty acids for weanling piglets raised in commercial farm[J].Veterinary Research Communications, 2024, 48(6).DOI:10.1007/s11259-024-10539-1.
5. Wei S , Amevor F K , Du X ,et al.Quercetin mitigates iron-induced cell death in chicken granulosa cell[J].Journal of Animal Science & Biotechnology, 2024, 15(1).DOI:10.1186/s40104-024-01118-0.

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.