ELK Biotechnology

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Total Anti- Oxidative Capability (T- AOC)
Assay Kit Instruction
(Spectrophotometric method)

Serial No: BC040 Pack：100T/50S 50T/25S

1.Reagent composition and preparation:

	Composition (R means Reagent)	100T/50S	50T/25S	Storage condition
R1	Colorless liquid	60ml ×2 bottles	60ml ×1 bottle	2 ~ 8 C
R2	White crystals	Powder×2 vial	Powder×1 vial	2 ~ 8 C
R2 working solution preparation: Add 120ml double distilled water in each vial, dissolve completely (this powder is difficult to dissolve, if you want to increase dissolving rate, please place it in 37C water bath.)				
R3	Yellow stock solution	5ml ×1 bottle	3ml ×1 bottle	2 ~ 8 C away from light
	Diluent	60ml ×1 bottle	30ml×1 bottle	2 ~ 8 C
R3 working solution preparation: Dilute stock solution with diluent at ratio of 1:19, please use this working solution soon after preparation.				
R4	Colorless thick liquid	24ml ×1 bottle	12ml×1 bottle	Room temperature
R5	Colorless liquid	24ml ×1 bottle	12ml×1 bottle	Room temperature
R5 freezes in cold days, when use, please place it in 37C water bath until it becomes limpid completely				

2. Operation procedure:

(1) Blood serum (or plasma) T-AOC assay (sample pretreatment follows Appendix)

① Operation table:

	Sample tube (ml)	Contrast tube (ml)
R1	1	1
Blood serum (or plasma)	a*	
R2 working solution	2	2
R3 working solution	0.5	0.5
Mix sufficiently by vortex, place in 37C water bath for 30 minutes		
R4	0.1	0.1
Blood serum (or plasma)		a*
Mix sufficiently, place for 10 minutes, transfer in cuvettes of 1cm light path, measure OD values of all tubes at 520nm (adjust zero by double distilled water).		

* **Referenced sample volume:** For blood serum (or plasma), a*=0 .1ml.

② **Calculation**

a. Unit definition: At 37℃, 0.01 reaction system OD value increasing per ml blood serum (or plasma) per minute is considered as 1 T-AOC unit (U).

b. Formula:

$$\text{T-AOC (U/ml blood serum)} = \frac{OD_U - OD_C}{0.01} \div 30 \times N \times n$$

ODU: Sample tube OD value.

ODC: Contrast tube OD value.

N : Reaction system dilution times (Total volume of reaction solution / Samplevolume)

n: Sample dilution times before assay

c. Example:

Take 0.1ml blood serum to measure T-AOC, adjust zero by distilled water, in results, ODC is 0.053, ODU is 0.131, calculate as follows:

$$\text{T-AOC (U/ml blood serum)} = \frac{0.131 - 0.053}{0.01} \div 30 \times \frac{3.7}{0.1} \times 1$$
$$= 0.078 \times 123.33$$
$$= 9.62 \text{U/ml blood serum}$$

(2) Tissue T-AOC assay: (Sample pretreatment follows appendix).

① **Operation table:**

	Sample tube(ml)	**Contrast tube(ml)**
R1	1	1
Homogenate	a*	
R2 working solution	2	2
R3 working solution	0.5	0.5
Mix sufficiently by vortex, place in 37℃ water bath for 30 minutes		
R4	0.2	0.2
Homogenate		a*
R5	0.2	0.2

Mix sufficiently, place for 10 minutes, transfer in cuvettes of 1cm light path, measure OD values of all tubes at 520nm (adjust zero by double distilled water).

* **Referenced sample volume:** For 10% tissue homogenate, a*=0 .1~0 .2ml.

② **Calculation**

a. Unit definition: At 37℃, 0.01 reaction system OD value increasing per mg tissue protein per minute is considered as 1 T-AOC unit (U).

b. Formula:

$$\text{T-AOC (U/mg protein)} = \frac{ODu - ODc}{0.01} \div 30 \times N \div Cprot$$

ODU: Sample tube OD value.

ODC: Contrast tube OD value.

N : Reaction system dilution times (Total volume of reaction solution/Sample volume)

Cprot : Sample protein concentration (mg/ ml)

c. Example:

Take 0.15ml rat lung tissue homogenate to measure T-AOC, adjust zero by double distilled water, in results, ODC is 0.050, ODU is 0.214, protein concentration is 10.5mg/ ml, calculate as follows:

$$\text{T-AOC (U/mg protein)} = \frac{0.214-0.050}{0.01} \div 30 \times \frac{4.05}{0.15} \div 10.5$$

$$= 0.164 \times 8.571$$

$$= 1.41\ \text{U/mg protein}$$

(3) Whole blood T-AOC assay: (Sample pretreatment follows appendix).

① **Operation table:**

	Sample tube (ml)	**Contrast tube (ml)**
R1	1	1
Blood sample	a*	
R2 working solution	2	2
R3 working solution	0.5	0.5
Mix sufficiently by vortex, place in 37℃ water bath for 30 minutes		

R4	0.1	0.1
Blood sample		a*
Mix sufficiently, place for 10 minutes, transfer in cuvettes of 1cm light path, measure OD values of all tubes at 520nm (adjust zero by double distilled water).		

* **Referenced sample volume:** For 1:9 diluted whole blood, a*=0 .05ml.

② **Calculation**

a. **Unit definition:** At 37℃, 0.01 reaction system OD value increasing per ml whole blood per minute is considered as 1 T-AOC unit (U).

b. Formula:

$$\text{T-AOC (U/ml whole blood)} = \frac{OD_U - OD_C}{0.01} \div 30 \times N \times n$$

ODU: Sample tube OD value.

ODC: Contrast tube OD value.

N : Reaction system dilution times (Total volume of reaction solution/Sampling volume)

n: Sample dilution times before assay

c. Example:

Take 0.05ml 1:9 diluted (by double distilled water) mouse fresh whole blood to measure T-AOC, adjust zero by distilled water, in results, ODC is 0.115, ODU is 0.213, calculate as follows:

$$\text{T-AOC (U/ml whole blood)} = \frac{0.213 - 0.115}{0.01} \div 30 \times \frac{3.65}{0.05} \times 10$$

$$= 238.467 \text{ U/ml whole blood}$$

(4) Convenient operation procedure

If your have too many samples to assay, then it is suggested to use convenient operation procedure.

Mixed reagent preparation: Mix R1, R2, R3 at ratio of 1 : 2 : 0.5, mixed reagent should be used soon after preparation.

1 **Convenient operation table for blood serum (or plasma) / whole blood T-AOC assay:**

	Contrast tube(ml)	Sample tube(ml)
Blood serum (or plasma)		a
Mixed reagent	3.5	3.5
Mix sufficiently by vortex, place in 37℃ water bath for 30 minutes		
R4	0.1	0.1
Blood serum (or plasma)	a	
Mix sufficiently, place for 10 minutes, transfer in cuvettes of 1cm light path, measure OD values of all tubes at 520nm (adjust zero by double distilled water).		

2

3 **Convenient operation table for tissue T-AOC assay:**

	Contrast tube(ml)	Sample tube(ml)
10% tissue homogenate		a
Mixed reagent	3.5	3.5
Mix sufficiently by vortex, place in 37℃ water bath for 30 minutes		
R4	0.2	0.2
10% tissue homogenate	a	
R5	0.2	0.2
Mix sufficiently, place for 10 minutes, transfer in cuvettes of 1cm light path, measure OD values of all tubes at 520nm (adjust zero by double distilled water).		

4. Assay principle:

Organism contains various antioxidative compounds which can reduce Fe3+ to Fe2+, Fe2+ can react with phenanthrolin to produce stable complex. It is able to calculate T-AOC by measuring OD values.

5. Assay significance:

There are close correlations between T-AOC of organism defense system and health level. This defense system includes 2 systems: enzymatic & nonenzymatic. In enzymatic system, microelements act as active centers for various enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), catalase (CAT), glutathione S-transferase (GST), etc. Nonenzymatic system mainly include vitamins, amino acids &

metalloproteins such as VE, carotene, Vc, cysteine, methionine, tryptophane, histidine, glucose, copper-protein, transferrin, lactoferrin, etc. There are 3 main pathways of antioxidation in defense system: (1) Eliminate free radicals and active oxygen in order to avoid lipid peroxidation; (2) Decompose peroxides to interrupt peroxidation chain; (3) Remove catalytic metal ions. There are synergetic effect, compensation & dependency between different components of defense system.

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Appendix: T-AOC sample pretreatment

1. Blood serum (or plasma) pretreatment:

Centrifugate at 3500rpm for 10 minutes, take supernatant for assay. Some anticoagulants may cause sediment or turbid. Heparin anticoagulated blood plasma and blood serum needn't centrifuge.

2. Tissue sample pretreatment:

Tissue homogenate preparation: Weigh tissue accurately, add 9 times physiological saline according to mass(g)-volume(ml) ratio of 1:9, make 10% tissue homogenate in icewater bath , centrifugate at 2500rpm for 10 minutes, take supernatant for assay. Use Coomassie brilliant blue to measure protein concentration.

3. Cultured cells pretreatment:

Cultured cell suspension preparation: Take cultured cells, digest, centrifugate, remove supernatant and keep sediment of cells. Use physiological saline or other homogenate media to make $10^7/cm^3$ suspension, then do disruption. These are 3 methods to disrupt cells: ① By homogenizer (motor-driven or hand-driven) ② By ultrasonication ③ Freeze-thaw for 3 times (Method ③ may disturb enzyme activity sometimes). Prepard cell suspension needn't centrifugation. Mix sufficiently before adding samples. Use Coomassie brilliant blue to measure protein concentration.

4. Culture cell supernatant and some body fluids can follow blood serum pretreatment, these samples can be added directly in general.

5. Whole blood pretreatment : Dilute 10 times by double distilled water, do pre-test to determine dilution times and sample volume.