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

Catalog No：E-BC-K042-M

Specification：48T(44 samples)/96T(92 samples)

Measuring instrument：Microplate reader(540-560 nm)

Detection range：1.46-82.89 U/mL

Elabscience® Hydroxyl Free Radical Scavenging Capacity Colorimetric Assay Kit (Fenton 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

Tel: 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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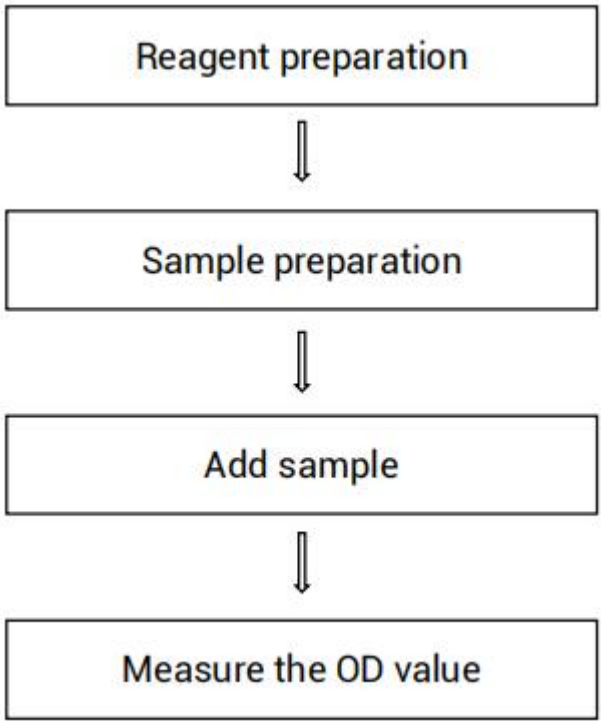
Assay summary



Intended use

This kit can be used to measure hydroxyl free radical scavenging capacity in serum, plasma and tissue samples.

Detection principle

The Fenton reaction is the most common chemical reaction that generates hydroxyl radicals (OH·). The amount of hydrogen peroxide is directly proportional to the amount of OH· produced by the Fenton reaction. After the electron accepter is given, adding griess reagent to produce the red substance, which is directly proportional to the amount of OH·. The substances contained in the sample that can eliminate hydroxyl free radical can consume OH·. The remaining OH· is detected to reflect hydroxyl free radical scavenging capacity.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Standard Stock Solution	0.5 mL × 1 vial	0.5 mL × 1 vial	2-8℃, 12 months
Reagent 2	Substrate Stock Solution	0.5 mL × 1 vial	0.5 mL × 1 vial	2-8℃, 12 months
Reagent 3	A Solution	0.8 mL × 1 vial	1.6 mL × 1 vial	2-8℃, 12 months shading light
Reagent 4	B Solution	6 mL × 1 vial	12 mL × 1 vial	2-8℃, 12 months
Reagent 5	Stop Solution	1.6 mL × 1 vial	3.2 mL × 1 vial	2-8℃, 12 months
Reagent 6	Chromogenic Agent A	16 mL × 1 vial	32 mL × 1 vial	2-8℃, 12 months shading light
Reagent 7	Chromogenic Agent B	16 mL × 1 vial	32 mL × 1 vial	2-8℃, 12 months shading light

	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

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 (540-560 nm, optimum wavelength: 550 nm), Incubator

Reagents:

Normal saline (0.9% NaCl), (2-8°C)

Reagent preparation

① Equilibrate all reagents to 25°C before use.

② The preparation of 9.79 mmol/L standard solution：
Before testing, please prepare sufficient 9.79 mmol/L standard solution according to the test wells. For example, prepare 1000 μL of 9.79 mmol/L standard solution (mix well 10 μL of standard stock solution and 990 μL of double distilled water). The prepared solution should be used up within 8 h.

③ The preparation of substrate stock working solution：
Before testing, please prepare sufficient substrate stock working solution according to the test wells. For example, prepare 1000 μL of substrate stock working solution (mix well 10 μL of standard stock solution and 990 μL of double distilled water). The prepared solution

should be used up within 8 h.

④ The preparation of A working solution：

Before testing, please prepare sufficient A working solution according to the test wells. For example, prepare 100 μL of A working solution (mix well 10 μL of A solution and 90 μL of double distilled water). The prepared solution should be used up within 8 h.

⑤ The preparation of reaction working solution：

For each tube, prepare 200 μL of reaction working solution (mix well 100 μL of A working solution and 100 μL of B solution). The prepared solution should be used up within 8 h.

⑥ The preparation of stop working solution：

Before testing, please prepare sufficient stop working solution according to the test wells. For example, prepare 100 μL of stop working solution (mix well 10 μL of stop solution and 90 μL of double distilled water). Store at 2-8°C for 3 days.

⑦ The preparation of chromogenic working solution：

For each tube, prepare 1000 μL of chromogenic working solution (mix well 500 μL of stop working solution, 250 μL of chromogenic agent A and 250 μL of chromogenic agent B). The prepared solution should be used up within 8 h.

⑧ Incubate substrate stock working solution and reaction working solution for

3 min at 37℃.

Sample preparation

① Sample preparation

Serum and plasma: detect directly.

Tissue sample:

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

② Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl), (2-8°C) with a dounce homogenizer at 4°C.

③ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M for animal tissue; E-BC-K168-M for plant tissue).

② Inhibition ratio of sample

Before the formal experiment, it needs to choose 2-3 samples for diluting a series of diluent and determine the dilution factor when the inhibition ratio is

20%~50% (the optimal inhibition ratio is the range of 40%~50%).

$$\text{Inhibition ratio} = \frac{A_{control} - A_{sample}}{A_{control} - A_{blank}} \times 100\%$$

③ Dilution of sample

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

Sample type	Dilution factor
Human serum	8-32
Human plasma	32-64
Mouse serum	16-32
10% Rat lung tissue homogenate	32-64

Note: The diluent is normal saline (0.9% NaCl), (2-8°C). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① Time accurately for reaction time with a stopwatch.
② Mix well quickly after adding the reaction working solution.
③ For ensuring the accuracy of the reaction time, it is better to measure no more than 3 samples at same time.

Operating steps

① Blank tube: add 200 μL of double distilled water into the 2 mL EP tubes.
Standard tube: add 100 μL of double distilled water into the 2 mL EP tubes.
Control tube: add 100 μL of double distilled water into the 2 mL EP tubes.
② Add 100 μL of 9.79 mmol/L standard solution into standard tubes.
Add 100 μL of 9.79 mmol/L substrate stock working solution into sample tubes and control tubes.
③ Add 100 μL of sample into sample tubes.
④ Add 200 μL of reaction working solution into each tube.
⑤ Keep time immediately after adding the reaction working solution, mix fully and accurately react 1 min at 37°C. Add 1 mL of chromogenic working solution into each tube to terminate the reaction.
⑥ Mix fully and take 200 μL of the solution into the corresponding wells. Stand for 20 min at room temperature. Measure the OD values of each well at 550 nm with microplate reader.

Calculation

The sample:

1. Serum (plasma) samples:

Definition: The amount of 1 mL serum (plasma) that consume 1 mmol/L substrate in reaction system in 1 min at 37℃ is defined as 1 unit of hydroxyl radical scavenging capacity.

$$\text{Hydroxyl free radical scavenging capacity (U/mL)} = \frac{A_{control} - A_{sample}}{A_{standard} - A_{blank}} \times 9.79* \div V \times f$$

2. Tissue samples:

Definition: The amount of 1 mg tissue protein that consume 1 mmol/L substrate in reaction system in 1 min at 37℃ is defined as 1 unit of hydroxyl radical scavenging capacity.

$$\text{Hydroxyl free radical scavenging capacity (U/mgprot)} = \frac{A_{control} - A_{sample}}{A_{standard} - A_{blank}} \times 9.79* \div (C_{pr} \times V) \times f$$

[Note]:

$A_{control}$: the OD value of control well.

A_{sample}: the OD value of sample well.

$A_{standard}$: the OD value of standard well.

A_{blank}: the OD value of blank well.

9.79*: Stand concentration, 9.79 mmol/L

V: the volume of sample added into the reaction system, mL.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, mgprot/mL

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 (U/mL)	2.94	8.82	16.17
%CV	5.4	2.2	3.3

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 (U/mL)	2.94	8.82	16.17
%CV	7.2	5.6	4.5

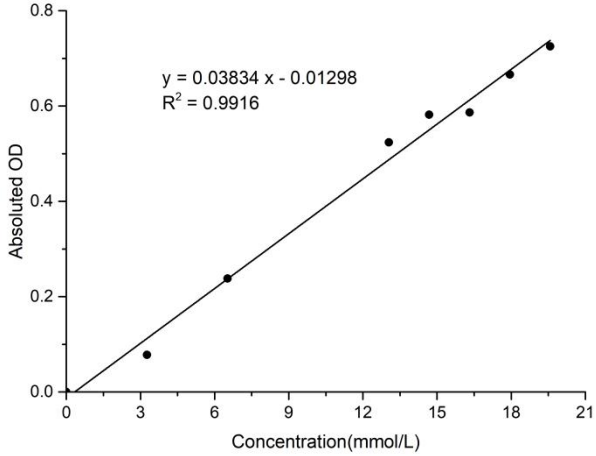
Sensitivity

The analytical sensitivity of the assay is 1.46 U/mL. 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)	**0.00**	**3.26**	**6.53**	**13.05**	**14.69**	**16.32**	**17.95**	**19.58**
OD	0.038	0.118	0.276	0.560	0.613	0.623	0.705	0.763
	0.039	0.115	0.277	0.564	0.628	0.627	0.704	0.764
Average OD	0.039	0.117	0.277	0.562	0.621	0.625	0.705	0.764
Absoluted OD	0.000	0.078	0.238	0.524	0.582	0.587	0.666	0.725



Appendix Π Example Analysis

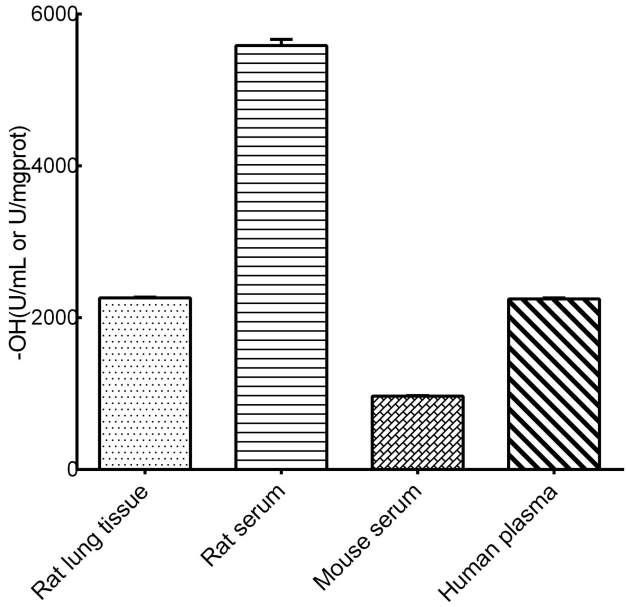
Example analysis：

Take 100 μL of human serum (dilute for 16 times), and carry the assay according to the operation steps. The results are as follows:

The OD value of the standard well is 0.375, the OD value of the control well is 0.569, the OD value of the sample well is 0.382, the OD value of the blank well is 0.039, and the calculation result is:

$$\text{hydroxyl free radical scavenging capacity (U/mL)} = \frac{0.569 - 0.382}{0.375 - 0.039} \times 9.79 \div 0.1 \times 16 = 871.78 \text{ U/mL}$$

Detect, 10% rat lung tissue homogenate (dilute for 32 times), rat serum (dilute for 150 times), mouse serum (dilute for 32 times), human plasma (dilute for 64 times) according to the protocol, the result is as follows：



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.