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

Catalog No：E-BC-K527-M

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

Measuring instrument：Microplate reader（500-530 nm）

Elabscience® Hydroxyl Free Radical Scavenging Capacity Assay Kit

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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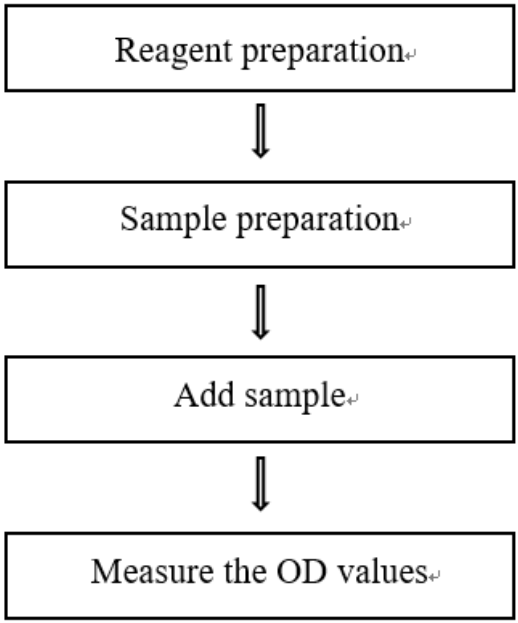
Assay summary



Intended use

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

Detection principle

H_2O_2/Fe^{2+} can generate hydroxyl free radical through Fenton reaction. Salicylic acid can effectively capture the generated hydroxyl radical and react with it to generate colored substances 2, 3-dihydroxybenzoic acid. There is a characteristic absorption peak at 510 nm. After adding the substances with scavenging ability, the colored substances are reduced, thus measuring the hydroxyl radical scavenging ability of the sample according to the value of absorption value.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Substrate A	Powder ×1 vial	Powder ×2 vials	2-8℃, 12 months shading light
Reagent 2	Substrate B	Powder ×1 vial	Powder ×2 vials	2-8℃, 12 months shading light
Reagent 3	Substrate C	12 mL ×1 vial	24 mL ×1 vial	2-8℃, 12 months shading light
	Microplate	96 wells		No requirement
	Plate Sealer	2 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:

Micropipettor, Vortex mixer, Centrifuge, Microplate reader (500-530 nm)

Reagents:

Double distilled water, Absolute ethanol

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of substrate A working solution：
Dissolve a vial of substrate A with 10 mL absolute ethanol (self-prepared), mix well. Store at 2-8 ℃ for 1 month protected from light.

③ The preparation of substrate B working solution：
Dissolve a vial of substrate B with 8 mL double distilled water. Store at 2-8 ℃ for 1 month protected from light.

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.

Tissue sample:

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

② Homogenize 20 mg tissue in 180 μL double distilled water with a dounce homogenizer at 4℃.

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

② Dilution of sample

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

Sample type	Dilution factor
Human serum	1
Dog serum	1
Rat serum	1
Cynomolgus monkey serum	1
10% Rat spleen tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Rat lung tissue homogenate	1

Note: The diluent is double distilled water. For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

① There should be no bubbles in the wells of the microplate when measuring the OD value.

② Before using pipette to take the reagent, balance the pipette tip using the reagent (slowly take the liquid, blow and beat repeatedly for three times).

Operating steps

① Control tube: Take 100 μL of substrate A working solution into 1.5 mL EP tube.

Sample tube: Take 100 μL of substrate A working solution into 1.5 mL EP tube.

Blank tube: Take 100 μL of substrate A working solution into 1.5 mL EP tube.

② Add 100 μL of substrate B working solution and 500 μL of double distilled water into control tubes.

Add 100 μL of substrate B working solution and 480 μL of double distilled water into sample tubes.

Add 600 μL of double distilled water into blank tubes.

③ Add 200 μL of substrate C into each tube.

④ Add 20 μL of sample into sample tubes.

⑤ Mix fully, incubate at 37℃ for 20 min. Take 200 μL of reaction solution into the corresponding wells and measure the OD values of each well at 510 nm with microplate reader. (Note: When there is turbidity in the sample tube, it will affect the result. Centrifuge at room temperature at 10000 g for 5 min, then take the supernatant for determination).

Calculation

The sample:

$$\text{Hydroxyl free radical scavenging capacity (\%)} = \frac{A_1 - A_3}{A_1 - A_2} \times 100\%$$

[Note]

A_1: $OD_{Contorl}$

A_2: OD_{Blank}

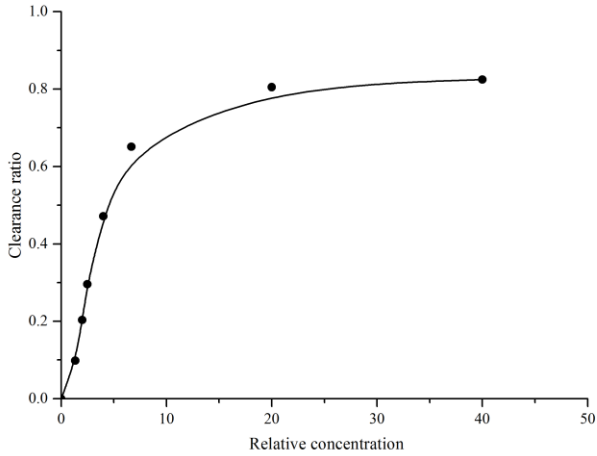
A_3: OD_{Sample}

Note: When the hydroxyl radical scavenging capacity of the sample is less than 10% or more than 50%, increase the sample added to the reaction appropriately or dilute the sample to ensure that the scavenging capacity is between 10-50%; In order to compare the hydroxyl radical scavenging capacity of different samples, decrease the volume of double distilled water in the reaction system while increasing the sample volume, so as to ensure the final reaction volume of the sample tube remains the same.

Appendix I Performance Characteristics

Standard curve:

Dilute human serum with double distilled water to different concentrations, then detect the hydroxyl free radical scavenging capacity, plot the standard curve and data are provided as below for reference only:



Appendix II Example Analysis

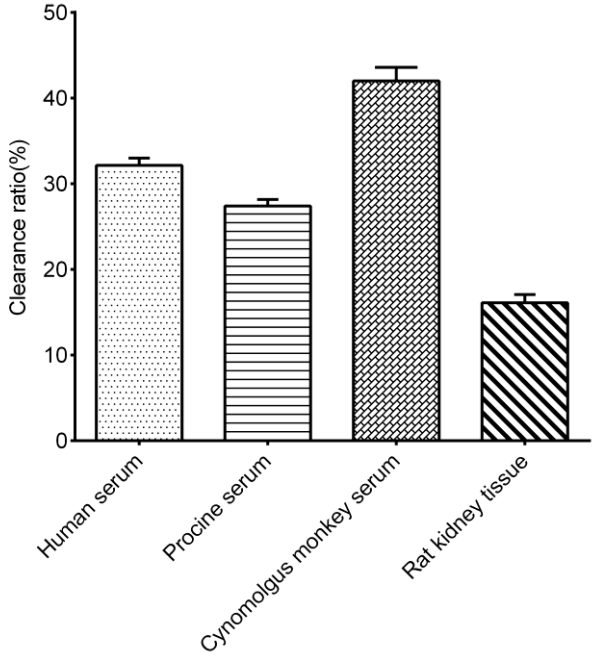
Example analysis：

Take 20 μL of human serum to corresponding wells and carry the assay according to the operation steps. The results are as follows:

the average OD value of the sample is 0.557, the average OD value of the blank is 0.038, the average OD value of the control is 0.803, and the calculation result is:

Hydroxyl free radical scavenging capacity (%) = (0.803-0.557) ÷ (0.803-0.038) ×100 = 32%

Detect human serum, porcine serum, cynomolgus monkey serum, 10% rat kidney tissue homogenate 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.