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Hydroxyl Free Radical Scavenging Capacity Assay Kit

Catalog No.：BC00023

Size：100T

If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉Email（Sale） order@enkilife.com

✉Email（Techsupport） techsupport@enkilife.com

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Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Hydroxyl Free Radical Scavenging Capacity Assay Kit
Detection Methods	Colorimetric
Sample type	Serum, plasma, tissue
Detection Type	Quantitative
Detection instrument and wavelength	Microplate reader (500-530 nm, optimal detection wavelength 510 nm)

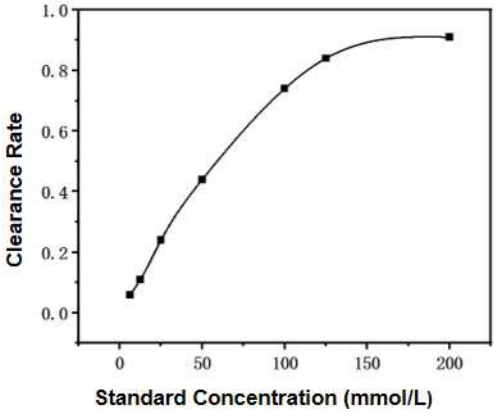
Product Introduction

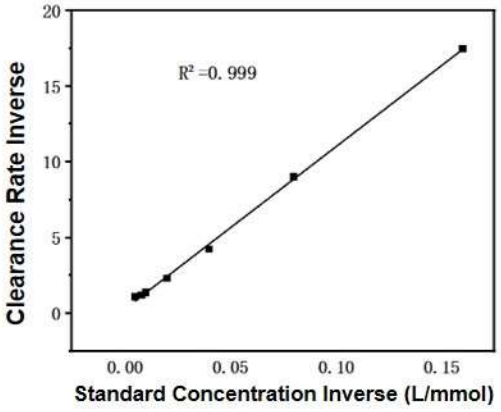
Hydroxyl free radical is a highly active oxygen free radical with a very high redox potential. It can react with a variety of molecules in the body, causing cell damage and dysfunction. Therefore, the generation and removal of hydroxyl free radicals play a vital role in maintaining the health of organisms and preventing and treating related diseases. Hydroxyl free radical scavenging ability detection has a wide range of application value as an important means to evaluate the effects of antioxidants, study antioxidant mechanisms, and develop new drugs.

Detection principle

H_2O_2/Fe^{2+} generates hydroxyl free radicals through Fenton reaction. Salicylic acid can effectively capture the generated hydroxyl free radicals and react with them to generate the colored substance 2,3-dihydroxybenzoic acid, which has a characteristic absorption peak at 510 nm. After adding substances with scavenging ability, the colored substance decreases, so the ability of the sample to scavenge hydroxyl free radicals can be judged based on the absorbance value.

The standard was diluted with distilled water at different times, and then its hydroxyl free radical elimination ability was measured. The curve was drawn as shown in the left figure below, and the linear comparison range was obtained: 10-50% (data is for reference only). The reciprocal of the standard concentration and the hydroxyl free radical elimination rate was obtained to obtain a linear relationship as shown in the right figure below.





Product Composition

Serial Number	Product Name	Packing Specifications (100T)	Storage
Reagent 1	Substrate A	30 mL	-20℃
Reagent 2	Substrate B	20 mL	-20℃, avoid light , store at 2-8℃ after opening , valid for three months.
Reagent 3	Substrate C	60 mL	-20℃
Reagent 4	Ascorbic acid standards	28 mg	-20℃
Consumables 1	96-well ELISA plate	1 plate	RT
Consumables 2	96-well membrane	2 pieces	RT

Storage

The unopened kit can be stored at -20℃ for 6 months. After opening, it can be stored at 2-8℃ for 3 months.

Preparation before the experiment

Sample processing

1. Serum samples: Direct assay.
2. Tissue samples: Take 0.020-0.1 g of fresh tissue blocks, rinse with 2-8°C PBS (0.01 M, pH 7.4) to remove blood, blot dry with filter paper, weigh, put into a homogenizer, add physiological saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) at a ratio of weight (g): volume (mL) = 1:9, and centrifuge at 4°C, 10000×g for 10 min. Take the supernatant and place it on ice for testing, and keep part of the supernatant for measurement.
3. Sample dilution: Generally, serum samples do not need to be diluted. Before the formal test of special samples, 2-3 samples with large expected differences can be selected and diluted into

different concentrations for preliminary experiments. Based on the results of the preliminary experiments and the clearance capacity of this kit (10-50%), dilution is performed. The diluent is distilled water.

· Preparation of the kit

Take out all reagents and return to room temperature before use.

Operation process

1. Standard curve determination

Take 0.8 ml of distilled water and add it to the ascorbic acid standard (28 mg). After fully dissolving, prepare a 200 mM ascorbic acid standard solution. The ascorbic acid standard solution should be prepared and used immediately.

The 200 mM ascorbic acid standard solution was diluted to 125, 100, 50, 25, 12.5, and 6.25 mM and measured according to the following steps.

2. Operation steps

blank control wells, standard wells, and sample wells in a 96-well plate .

	Control group	Blank group	sample
Reagent 1 (μl)	100	100	100
Reagent 2 (μl)	100	—	100
Distilled water (μl)	500	600	480
Reagent 3 (μl)	200	200	200
Sample to be tested (μl)	—	—	20
Gently shake to mix, incubate at 37°C for 20 min, take 200 μl into the corresponding wells of a 96-well ELISA plate, and measure the OD value of each well at a wavelength of 510 nm.			

Note: If the test tube is turbid, it will affect the test results. Centrifuge at 10,000 × g for 5 min at room temperature and then take the supernatant for test.

Result calculation

$$\text{Hydroxyl Free Radical Scavenging Capacity (\%)} = \frac{A_1 - A_3}{A_1 - A_2} \times 100\%$$

Annotation:

A1: OD value of control well

A2: Blank well OD value

A3: Determine the OD value of the well

Note: When the hydroxyl radical scavenging capacity of the sample is below 10% or above 50%, the sample loading amount can be appropriately increased or the sample can be diluted to ensure that the scavenging capacity is between 10-50%; in order to compare the hydroxyl radical scavenging capacity of different samples, while increasing the loading amount, the amount of double distilled water added to the reaction system can be reduced to ensure that the final reaction volume of the assay tube remains unchanged.

Notes

1. The optimal detection wavelength of the ELISA instrument is 510 nm, and detection can be performed in the range of 500 nm - 530 nm .
2. This product is limited to scientific research by professionals and must not be used for clinical diagnosis or treatment, used as food or medicine, or stored in ordinary residences.