Technical Manual

Hydroxyl Free Radical Scavenging Capacity Assay Kit

- Catalogue Code: MAES0187
- Size: 96T
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Storage:

2-8°C for 3 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 3 months.

Do not use components from different batches of kit.

2. Background

Hydroxyl radical is a kind of reactive oxygen species, which can kill red blood cells, degrade DNA, cell membrane and polysaccharide compounds, causing damage to cell structure and function, and then leading to metabolic disorders in the body to cause diseases. The scavenging ability of hydroxyl free radical is one of the important indexes of the antioxidant ability of samples. It has been widely used in the research of antioxidant health care products and drugs.

3. Intended Use

This kit can be used for detection of hydroxyl free radical scavenging capacity in serum, plasma and tissue samples.

4. 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.

5. Kit Components & Storage

Item	Specification	Storage
Substrate A	Lyophilized × 2 vials	2-8°C, 3 months, avoid direct sunlight
Substrate B	Lyophilized × 2 vials	2-8°C, 3 months, avoid direct sunlight
Substrate C	24 mL × 1 vial	2-8°C, 3 months, avoid direct sunlight
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Vortex mixer
- centrifuge
- Microplate reader (500-530 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Absolute ethanol

6. Assay Notes:

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).

7. Reagent Preparation:

1. Bring all the reagents to room temperature before use.

2. Preparation of **Substrate A working solution:** Dissolve substrate A with 10 mL of absolute ethanol (self-prepared), mix fully and store it at 2-8°C and avoid direct sunlight for 1 month.

3. Preparation of **Substrate B working solution:** Dissolve substrate B with 8 mL of double distilled water, mix fully and store it at 2-8°C and avoid direct sunlight for 1 month.

8. Sample Preparation

1. Serum sample:
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) and preserve on ice before detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of double distilled water (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and and the scavenging capacity detection range (10-50%).

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.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 510 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85
B	B	B	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86
C	S1	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
D	S2	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
E	S3	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
F	S4	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
G	S5	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
H	S6	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92

Note: A, control wells; B, blank wells; S1-S92, sample wells.

10. Operation Steps

The measurement of samples

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.

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

4. Add 600 μL of double distilled water into blank tubes.

5. Add 200 μL of substrate C into each tube.

6. Add 20 μL of sample into sample tubes.

7. Mix fully, incubate at 37°C 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).

Operation Table

Enzymatic reaction

	Control tube	Blank tube	Sample tube
Substrate A working solution (μL)	100	100	100
Substrate B working solution (μL)	100		100
Double distilled water (μL)	500	600	480
Substrate C (μL)	200	200	200
Sample (μL)			20
Mix fully, incubate at 37°C 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.			

11. Calculations

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

A_1: $OD_{Contorl}$
A_2: OD_{Blank}
A_3: OD_{Sample}

12. Performance Characteristics

Average inter-assay CV (%)	4.6
Average intra-assay CV (%)	4.1

Analysis

Take 20 µL of human serum to corresponding wells and carry the assay according to the operation table.

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:

$$\textbf{Hydroxyl free radical scavenging capacity (\%)} = \frac{\textbf{(0.803 - 0.557)}}{\textbf{(0.803 - 0.038)}} \times 100\%$$

$$= 32\%$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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