TECHNICAL MANUAL

Hydroxyl Free Radical Scavenging Capacity Assay Kit

- **SKU CODE:** MAES0187
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add reagents to tubes
- Add sample and incubate
- Measure absorbance at 510 nm
- Calculate hydroxyl free radical scavenging capacity

2. Intended use

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

3. Detection principle

H_{2O}_2/Fe^2+ can generate hydroxyl free radicals through the Fenton reaction. Salicylic acid can effectively capture the generated hydroxyl radicals and react with them to generate colored substances (2,3-dihydroxybenzoic acid). There is a characteristic absorption peak at 510 nm. After adding substances with scavenging ability, the colored substances are reduced, thus measuring the hydroxyl radical scavenging ability of the sample according to the absorption value.

4. Kit components & storage

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

5. Materials prepared by users

Instruments:

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

Reagents:

Double distilled water, Absolute ethanol

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of Substrate A working solution:** Dissolve one vial of Substrate A with 10 mL absolute ethanol (self-prepared), mix well. Store at 2-8 °C for 1 month protected from light.
3. **Preparation of Substrate B working solution:** Dissolve one vial of Substrate B with 8 mL double distilled water. Store at 2-8 °C for 1 month protected from light.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80 °C 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 °C.

3. Centrifuge at 10,000 x g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

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 perform a pretest to confirm the dilution factor.

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

9. Operating steps

1. **Tube preparation:** 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 reagents:** 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 °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 10,000 x g for 5 min, then take the supernatant for determination).

10. Calculation

The sample:

Hydroxyl free radical scavenging capacity (%) = $(A_1 - A_3) / (A_1 - A_2) \times 100\%$

[Note]

A_1: $OD_{Control}$

A_2: OD_{Blank}

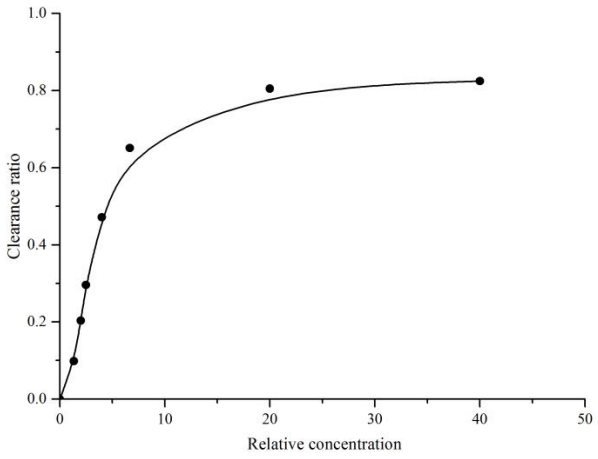
A_3: OD_{Sample}

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.

11. 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:



12. Appendix II Example Analysis

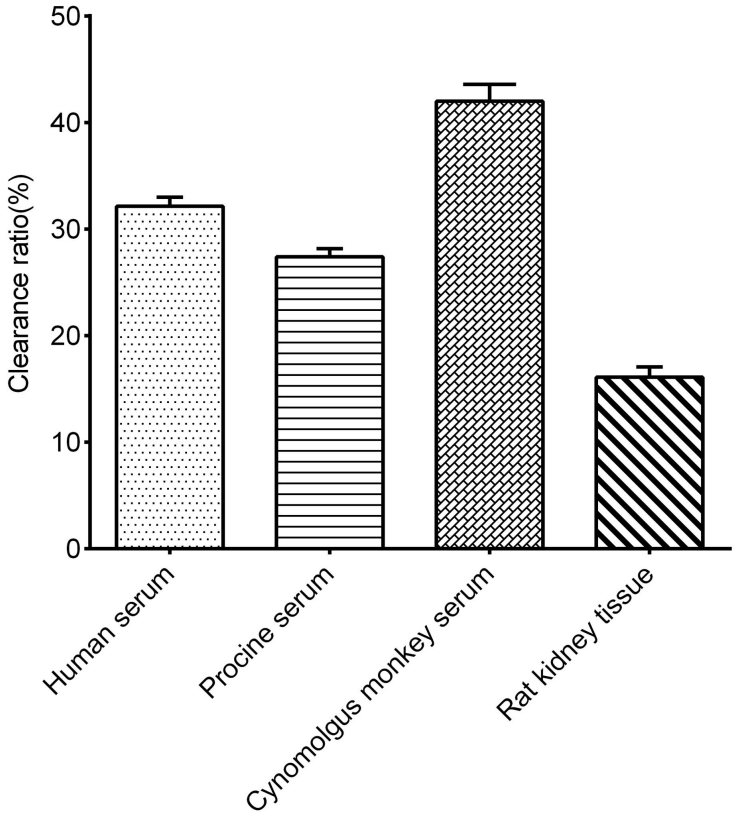
Example analysis:

Take 20 μL of human serum to corresponding wells and carry out 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) x 100 = 32%

Detect human serum, porcine serum, cynomolgus monkey serum, 10% rat kidney tissue homogenate according to the protocol, the result is as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.