Hydroxyl Free Radical (HO) Scavenging Capacity Assay Kit

Catalog No.: abx295105

Size: 96 tests

Detection Range: 10% HO Scavenging Capacity – 50% HO Scavenging Capacity

Sensitivity: 10% HO Scavenging Capacity

Storage: Store all components at 4°C. Store all components in the dark.

Application: For detection and quantification of Hydroxyl Free Radical scavenging capacity in serum, plasma, and tissue homogenates.

Introduction

Hydroxyl Free Radicals (HO) are a specific kind of Reactive Oxygen Species (ROS), which are generated in most cells by respiration. All reactive oxygen species are capable of damaging cell membranes, degrading DNA, and interrupting normal metabolic processes, potentially leading to disease or oncogenesis. As such, the ability of samples to scavenge and remove Hydroxyl Free Radicals can be taken as a general indicator of cell health, making it a useful measure for researchers investigating a variety of different diseases with complex pathogenesis and progressions.

Abbexa's Hydroxyl Free Radical (HO) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Hydroxyl Free Radical scavenging capacity. When mixed, H_2O_2 and Fe^{2+} ions react to produce hydroxyl free radicals (Fenton's reaction). Salicylic acid will react with these radicals to produce 2,3-dihydrobenzoic acid, a colored compound with an absorbance maximum at 510 nm. The scavenging of free radicals by the tested samples will reduce this color intensity. The intensity of the color is thus inversely proportional to the sample's Hydroxyl Free Radical scavenging capacity, which can then be calculated.

Kit components

1. 96-well microplate
2. Substrate A: 2 vials
3. Substrate B: 2 vials
4. Substrate C: 24 ml
5. Plate sealer: 2

Materials required but not provided

1. Microplate reader (510 nm)
2. Double-distilled water
3. Absolute (anhydrous) ethanol
4. 1.5 ml microcentrifuge tubes
5. Centrifuge
6. Vortex mixer
7. Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum** and **Plasma:** Serum and plasma samples can be tested directly.
- **Tissue Homogenates:** Carefully weigh out at least 20 mg of tissue, and add into ice-cold double-distilled water in a ratio of 1 : 9 weight (mg) to volume (µl) (e.g. for 20 mg of tissue, add into 180 µl double-distilled water). Homogenize manually, using a mechanical homogenizer or by ultrasonication, at 4°C. Collect the tissue homogenate, and centrifuge at 10,000 × g for 10 minutes at 4°C. Collect the supernatant and assay immediately, or aliquot and store at -80°C for up to 1 month. Keep on ice until detection.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with double-distilled water, then carry out the assay procedure. 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
10% Rat kidney tissue homogenate	1
10% Rat lung tissue homogenate	1
10% Rat spleen tissue homogenate	1
10% Mouse liver tissue homogenate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Substrate A Working Solution:** Dissolve 1 vial of Substrate A with 10 ml of absolute (anhydrous) ethanol. Mix well, until the powder has fully dissolved. The Substrate A Working Solution can be stored for up to 1 month at 4°C in the dark.
- **Substrate B Working Solution:** Dissolve 1 vial of Substrate B with 8 ml double-distilled water. Mix well, until the powder has fully dissolved. The Substrate B Working Solution can be stored for up to 1 month at 4°C in the dark.

Note:

- Allow all reagents to equilibrate to room temperature before use.

B. Assay Procedure

Pre-heat the incubator and ensure it has reached a stable temperature before use.

When plating, pipette slowly to avoid bubbles. Ensure that there are no bubbles in the wells during OD measurement.

1. Mark microcentrifuge tubes for each sample, control, and blank. Each sample requires a corresponding control. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 100 µl of Substrate A Working Solution to all tubes.
3. Add 100 µl of Substrate B Working Solution to the sample and control tubes.
4. Add 480 µl of double-distilled water to the sample tubes.
5. Add 500 µl of double-distilled water to the control tubes.
6. Add 600 µl of distilled water to the blank tubes.
7. Add 200 µl of Substrate C to all tubes.
8. Add 20 µl of sample to the sample tubes. All tubes should now contain 900 µl of total liquid.
9. Mix fully, and then incubate all tubes at 37°C for 20 minutes.
10. Mark positions on the 96-well microplate well for each sample, control, and blank.
11. Take 200 µl of solution from each tube, and transfer to the corresponding wells on the 96-well microplate.
12. Measure the OD of each well with a microplate reader at 510 nm.

Note:

- If there is a cloudy suspension in the tubes after incubating at 37°C for 20 minutes (Step 9.), centrifuge the affected tubes at 10,000 × g for 5 minutes. Plate the clear supernatant for Step 12.

C. Calculation of Results

1. All samples:

$$\text{HO Free Radical Scavenging Capacity (\%)} = \frac{\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}}}{\text{OD}_{\text{Control}} - \text{OD}_{\text{Blank}}} \times 100$$

where:

$\text{OD}_{\text{Control}}$	OD value of control
$\text{OD}_{\text{Sample}}$	OD value of sample
OD_{Blank}	OD value of blank

Note:

- For optimal results, the Hydroxyl Free Radical Scavenging Capacity should be in the range 10% – 50%.
- If the sample scavenging capacity is < 10%:
    - Repeat sample analysis, but increase the volume of sample added in Section B. Step 8. Proportionally decrease the volume of double-distilled water added in Section B. Step 4. to ensure the final volume in the tube is 900 µl.
- If the sample scavenging capacity is > 50%:
    - Repeat sample analysis, but decrease the volume of sample added in Section B. Step 8. Proportionally increase the volume of double-distilled water added in Section B. Step 4. to ensure the final volume in the tube is 900 µl.

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.