Superoxide Anion Assay Kit

Catalog No.: abx298898

Size: 96 tests

Storage: Store the Enzyme stock solution and Standard in the dark at -20°C and the rest of the components in the dark at 4°C for up to 6 months.

Application: For detection and quantification of superoxide anion inhibition or production activity in serum, plasma, tissue, cell lysates, cell culture supernatants, urine, and other biological fluids

Introduction

Superoxide anion free radicals are a type of reactive oxygen, which is formed by the reduction of molecular oxygen. Excessive accumulation of reactive oxygen species will lead to oxidative stress. Superoxide anion free radicals are produced through the reaction system of xanthine and xanthine oxidase. WST-1, a water-soluble tetrazolium salt, can react with the generated superoxide anion to produce water-soluble formazan. Samples containing superoxide anion free radical inhibitors such as Vitamin C can inhibit the formation of formazan. Samples containing a substance that produces superoxide anion free radical, it can promote the formation of formazan dye. By colorimetric analysis of WST-1 products, the units of activity of inhibition or production of superoxide anion radical in samples can be tested.

Abbexa's Superoxide Anion Assay Kit is a quick, convenient, and sensitive method for measuring and calculating superoxide anion inhibition or production activity. The formazan product has an absorbance maxima at 450 nm. The intensity of the color is proportional to the superoxide anion inhibition activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Buffer solution: 24 ml
3. Substrate solution: 0.14 ml
4. Enzyme stock solution: 0.3 ml
5. Enzyme diluent: 2 × 1.5 ml
6. Vitamin C standard: 3 vials
7. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (450 nm)
2. Distilled water
3. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Vials/tubes
6. Sonicating water bath
7. Centrifuge
8. Vortex mixer
9. 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:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge for 10 mins at 700-1000 × g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Weigh 0.02-1 g of tissue and wash with pre-chilled PBS. For each 1 g of tissue, add 9 ml of pre-chilled normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 1500 x g at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately.
- **Cell lysates:** Collect cells into a centrifuge tube and wash with PBS. Centrifuge at 1000 × g for 10 min and discard the supernatant. Add 300-500 µl of normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) per 1 × 10^6 cells, then sonicate in an ice water bath. Centrifuge at 10,000 × g at 4 °C for 10 min. Take the supernatant into a new centrifuge tube (while kept on ice) and analyze immediately. The protein concentration in the supernatant should be determined separately.
- **Urine:** Collect fresh urine into a sterile container, then centrifuge at 10,000 × g at 4°C for 15 min. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.

Samples should not contain detergents such as SDS, Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

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 normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure and calculate the inhibition ratio of the samples. The optimal dilution factor is an inhibition ratio in the range of 40% - 60%, where the inhibition ratio can be calculated as:

$$\text{Inhibition Ratio (\%)} = \frac{(\text{OD}_{\text{Control}} - \text{OD}_{\text{ControlBlank}}) - (\text{OD}_{\text{Sample}} - \text{OD}_{\text{SampleBlank}})}{\text{OD}_{\text{Control}} - \text{OD}_{\text{ControlBlank}}} \times 100$$

If the inhibition ratio is > 65%, the sample should be diluted further. If the inhibition ratio is < 30%, the sample concentration should be increased.

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

Sample Type	Dilution Factor
Human Serum	4-7
Mouse Serum	15-25
Rat Serum	25-35
Human Saliva	1
HepG2 Culture Supernatant	1
10% Rat Brain Tissue Homogenate	150-200
10% Rat Liver Tissue Homogenate	500-600
10% Mouse Liver Tissue Homogenate	500-600
10% Mouse Heart Tissue Homogenate	150-200
10% *Epipremnum aureum* Tissue Homogenate	20-30

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 working solution:** Mix the Buffer solution and the Substrate solution at a ratio of 200:1. Prepare immediately before carrying out the assay. Unused Substrate working solution can be stored at 2-8°C for up to 7 days.
- **Enzyme working solution:** Mix the Enzyme stock solution and Enzyme diluent at a ratio of 1:10 on ice. Prepare immediately before carrying out the assay. Unused Enzyme working solution can be stored at 2-8°C for up to 3 days.
- **5 mg/ml standard solution:** Dissolve a vial of Vitamin C Standard with 1 ml distilled water. Prepare immediately before carrying out the assay and mix fully.
- **0.05 mg/ml standard solution**: Dilute the 5 mg/ml standard solution 100-fold with distilled water. Prepare immediately before carrying out the assay and mix fully. This solution is readily oxidized, it is therefore recommended to use within 30 minutes of preparation.

B. Assay Procedure

1. Set control, control blank, standard, standard blank, sample and sample blank wells on the microplate and record their positions. It is recommended to use 2 control, 2 control blank, 2 standard and 2 standard blank wells for each assay run. Each sample requires a sample blank well. *Add the solution to the bottom of each well without touching the side walls. Pipette samples up and down to mix before adding to wells. Avoid foaming or bubbles.*

2. Add 20 µl of 0.05 mg/ml standard solution to the standard and standard blank wells.
3. Add 20 µl of distilled water to the control and control blank wells.
4. Add 20 µl of sample to each of the sample and sample blank wells.
5. Add 20 µl of enzyme working solution to the control, standard and sample wells.
6. Add 20 µl of enzyme diluent to the control blank, standard blank and sample blank wells.
7. Add 200 µl of substrate working solution to all wells.
8. Gently tap the plate to mix, or use a microplate shaker. Incubate at 37°C for 20 minutes.
9. Measure the OD of each well with a microplate reader at 450 nm.

C. Calculation of Results

Inhibition calculations:

1. Serum, plasma, urine, and cell culture supernatant samples:

One unit of superoxide anion inhibition activity is defined as the quantity of superoxide anion radical inhibited by 1 L of sample in 20 minutes at 37°C that is equivalent to the inhibition by 1 mg of Vitamin C.

$$\text{Inhibition of } O_2^{\cdot-}\ (\text{U/L}) = \frac{(OD_{\text{Control}} - OD_{\text{ControlBlank}}) - (OD_{\text{Sample}} - OD_{\text{SampleBlank}})}{(OD_{\text{Control}} - OD_{\text{ControlBlank}}) - (OD_{\text{Standard}} - OD_{\text{StandardBlank}})} \times C_{\text{Standard}} \times 1000 \times f$$

2. Tissues and cell lysate samples:

One unit of superoxide anion inhibition activity is defined as the quantity of superoxide anion radical inhibited by 1 g of protein in the sample in 20 minutes at 37°C that is equivalent to the inhibition by 1 mg of Vitamin C.

$$\text{Inhibition of } O_2^{\cdot-}\ (\text{U/g}) = \frac{(OD_{\text{Control}} - OD_{\text{ControlBlank}}) - (OD_{\text{Sample}} - OD_{\text{SampleBlank}})}{(OD_{\text{Control}} - OD_{\text{ControlBlank}}) - (OD_{\text{Standard}} - OD_{\text{StandardBlank}})} \times \frac{C_{\text{Standard}} \times 1000 \times f}{C_{\text{Protein}}}$$

Production calculations:

1. Serum, plasma, urine and cell culture supernatant samples:

One unit of superoxide anion production activity is defined as the quantity of superoxide anion radical produced by 1 L of sample in 20 minutes at 37°C that is equivalent to the inhibition by 1 mg of Vitamin C.

$$\text{Production of } O_2^{\cdot-}\ (\text{U/L}) = \frac{(OD_{\text{Sample}} - OD_{\text{SampleBlank}}) - (OD_{\text{Control}} - OD_{\text{ControlBlank}})}{(OD_{\text{Control}} - OD_{\text{ControlBlank}}) - (OD_{\text{Standard}} - OD_{\text{StandardBlank}})} \times C_{\text{Standard}} \times 1000 \times f$$

2. Tissues and cell lysate samples:

One unit of superoxide anion production activity is defined as the quantity of superoxide anion radical produced by 1 g of protein in the sample in 20 minutes at 37°C that is equivalent to the inhibition by 1 mg of Vitamin C.

$$\text{Production of } O_2^{\cdot-}\ (\text{U/g}) = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{SampleBlank}}) - (\text{OD}_{\text{Control}} - \text{OD}_{\text{ControlBlank}})}{(\text{OD}_{\text{Control}} - \text{OD}_{\text{ControlBlank}}) - (\text{OD}_{\text{Standard}} - \text{OD}_{\text{StandardBlank}})} \times \frac{\text{C}_{\text{Standard}} \times f}{\text{C}_{\text{Sample}}}$$

where:

$\text{OD}_{\text{Control}}$	OD value of control
$\text{OD}_{\text{ControlBlank}}$	OD value of control blank
$\text{OD}_{\text{Standard}}$	OD value of standard
$\text{OD}_{\text{StandardBlank}}$	OD value of standard blank
$\text{OD}_{\text{Sample}}$	OD value of sample
$\text{OD}_{\text{SampleBlank}}$	OD value of sample blank
$\text{C}_{\text{Standard}}$	Concentration of the standard (0.05 mg/ml)
$\text{C}_{\text{Protein}}$	Concentration of protein in sample
C_{Sample}	The concentration of sample (g/L)
1000	Unit conversion: 1 L = 1000 ml
f	The dilution factor of sample