Superoxide Dismutase (SOD) Assay Kit

Catalog No.: abx294018

Size: 96 tests

Detection range: 2.03 U/ml – 155 U/ml

Sensitivity: 2.03 U/ml

Storage: Store the Enzyme stock solution (20X) at -20°C and the rest of the components in the dark at 4°C.

Application: For detection and quantification of SOD 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. Superoxide Dismutase (SOD) catalyses the conversion of superoxide anions to O_2 through the reduction of Hydroxylamine to form Nitrite anions. Two types of SOD exist in animals, CuZn SOD and Mn SOD. Sample pretreatment with Extraction Solution selectively inhibits Mn SOD activity, allowing for the detection of CuZn SOD activity alone. The difference between Total SOD activity and CuZn SOD activity can be used to calculate the activity of Mn SOD. Reduction of the detection reagents by Nitrite form a coloured compound. By colorimetric analysis of reduction products, the units of activity of SOD in samples can be tested.

Abbexa's SOD Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Total SOD, CnZn SOD and Mn SOD activity. The reaction products have an absorbance maxima at 550 nm. The intensity of the color is proportional to SOD activity, which can then be calculated.

Kit components

1. Buffer solution: 12 ml
2. Nitrogen Reagent: 12 ml
3. Substrate solution: 12 ml
4. Enzyme stock solution (20X): 0.6 ml
5. Enzyme diluent: 12 ml
6. Detection Reagent A: 1 vial
7. Detection Reagent B: 1 vial
8. Detection Reagent C Solution: 6 ml
9. Extraction solution: 25 ml

Materials Required But Not Provided

1. Spectrophotometer (550 nm)
2. Double distilled water
3. Normal saline (0.9% NaCl)
4. Pipette and pipette tips
5. Vials/tubes
6. Sonicating water bath
7. Centrifuge
8. Vortex mixer
9. Incubator

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Protocol

A. Preparation of samples and reagents

1. Reagents

- **Buffer Working Solution:** Dilute the Buffer Solution with double distilled water 10-fold (for example, add 108 ml of double distilled water to 12 ml of Buffer Solution to prepare 120 ml of Buffer Working Solution). Unused Buffer Working Solution can be stored at 2-8°C for up to 3 months.
- **Enzyme Working Solution:** Dilute the Enzyme stock solution (20X) with Enzyme diluent 20-fold on ice (for example, add 11.4 ml of Enzyme diluent to 0.6 ml of Enzyme stock solution (20X) to prepare 12 ml of Enzyme Working Solution). Prepare immediately before carrying out the assay. Unused Enzyme Working Solution can be stored at 2-8°C for up to 3 days.
- **Detection Reagent A Solution:** Preheat 90 ml of double distilled water to 70-80°C. Dissolve a vial of Detection Reagent A with preheated double distilled water to a final volume of 90 ml. Prepare immediately before carrying out the assay and mix fully. Unused Detection Reagent A Solution can be stored at 2-8°C in the dark for up to 3 months.
- **Detection Reagent B Solution:** Dissolve a vial of Detection Reagent B with double distilled water to a final volume of 90 ml. Prepare immediately before carrying out the assay and mix fully. Unused Detection Reagent B Solution can be stored at 2-8°C in the dark for up to 1 month.
- **Detection Reagent Working Solution:** Mix Detection Reagent A Solution, Detection Reagent B Solution, and Detection Reagent C Solution at a ratio of 3:3:2 (for example, mix 90 ml of Detection Reagent A Solution, 90 ml of Detection Reagent B Solution, and 60 ml of Detection Reagent C Solution to prepare 240 ml of Detection Reagent Working Solution. Prepare immediately before carrying out the assay and mix fully. Unused Detection Reagent Working Solution can be stored at 2-8°C in the dark for up to 1 month.

2. Sample Collection

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.

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- **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), 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 25% - 45%, where the inhibition ratio can be calculated as:

$$\text{T SOD inhibition (\%)} = \frac{(\text{OD}_{\text{T SOD Control}} - \text{OD}_{\text{T SOD Sample}})}{(\text{OD}_{\text{T SOD Control}} - \text{OD}_{\text{T SOD Blank}})} \times 100$$

$$\text{CuZn SOD inhibition (\%)} = \frac{(\text{OD}_{\text{CuZn SOD Control}} - \text{OD}_{\text{CuZn SOD Sample}})}{(\text{OD}_{\text{CuZn SOD Control}} - \text{OD}_{\text{CuZn SOD Blank}})} \times 100$$

If the inhibition ratio is > 55%, the sample should be diluted further. If the inhibition ratio is < 15%, the sample concentration should be increased.

Where dilutions are required, dilute with Normal Saline (0.9% NaCl). The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor	Volume added to the reaction (µl)
Mouse Serum	3-5	20-30
Human Serum	1	30-40
Rat Serum	3-5	20-30
Rat plasma	1-2	20-30
Human Hydrothorax	1	30-50
10% Mouse Liver Tissue Homogenate	50-80	20-30
10% Mouse Brain Tissue Homogenate	8-12	20-30
10% Mouse Kidney Tissue Homogenate	10-20	20-30
Human Urine	1	30-50
HepG2 cells (protein concentration 5.2 mg/ml)	5-10	20-30

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3. Sample Pretreatment

- **CuZn SOD Sample**: Add 0.1 ml of sample to 0.1 ml of Extraction Solution. Mix thoroughly with a vortex for 1 minute. Centrifuge at 3500 × g for 15 minutes. Take the supernatant for the CuZn SOD Assay.
- **CuZn SOD Control:** Add 0.1 ml of normal saline to 0.1 ml of Extraction Solution. Mix thoroughly with a vortex for 1 minute. Centrifuge at 3500 × g for 15 minutes. Take the supernatant for the CuZn SOD Assay.
- ***Total SOD (T SOD)*** *sample is the untreated sample, Extraction Solution is not added.*

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.
- **EDTA is not suitable for use with this kit. If an anticoagulant is required, it is recommended to use Heparin.**

B. Assay Procedure

1. Set CuZn SOD control, CuZn SOD sample; T SOD control, and T SOD sample tubes and record their positions.
2. Add 1 ml of Buffer Working Solution to all tubes.
3. Add 20-50 µl of T SOD Sample to the T SOD Sample tube.
4. Add 20-50 µl of double distilled water to the T SOD Control tube.
5. Add 20-50 µl of CuZn SOD Sample to the CuZn SOD Sample tube.
6. Add 20-50 µl of double distilled water to the CuZn SOD Control tube.
7. Add 0.1 ml of Nitrogen Reagent, 0.1 ml of Substrate solution and 0.1 ml of Enzyme Working Solution
8. Mix fully with a vortex, and incubate at 37°C for 40 minutes. If the room temperature is below 20°C, incubate for 45 minutes.
9. Add Detection Reagent Working Solution to all tubes.
10. Mix fully with an orbital shaker for 10 seconds. Allow to stand for 10 minutes at room temperature.
11. Calibrate the spectrophotometer to zero with double distilled water.
12. Measure the OD of each tube with the spectrophotometer at 550 nm.

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C. Calculation of Results

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

One unit of activity (U) is defined as the activity where the SOD inhibition ratio (1 ml reaction system) is equal to 50%.

$$\text{T SOD activity (U/ml)} = i_{\text{T SOD}} \times \frac{1}{50} \times \frac{V_1}{V_2} \times f = \frac{(\text{OD}_{\text{T SOD Control}} - \text{OD}_{\text{T SOD Sample}})}{(\text{OD}_{\text{T SOD Control}} - \text{OD}_{\text{T SOD Blank}})} \times \frac{100}{50} \times \frac{V_1}{V_2} \times f$$

$$\text{CuZn SOD activity (U/ml)} = i_{\text{CnZn SOD}} \times \frac{1}{50} \times \frac{V_1}{V_2} \times f = \frac{(\text{OD}_{\text{CuZn SOD Control}} - \text{OD}_{\text{CuZn SOD Sample}})}{(\text{OD}_{\text{CuZn SOD Control}} - \text{OD}_{\text{CuZn SOD Blank}})} \times \frac{100}{50} \times \frac{V_1}{V_2} \times f$$

$$\text{Mn SOD activity (U/ml)} = \text{T SOD activity} - \text{CuZn SOD activity}$$

2. Tissues and cell lysate samples:

One unit of activity (U) is defined as the activity where the SOD inhibition ratio (1 mg tissue, 1 ml reaction system) is equal to 50%.

$$\text{T SOD activity (U/ml)} = i_{\text{T SOD}} \times \frac{1}{50} \times \frac{V_1}{V_2} \times \frac{f}{C_p} = \frac{(\text{OD}_{\text{Total Control}} - \text{OD}_{\text{Total Sample}})}{(\text{OD}_{\text{Total Control}} - \text{OD}_{\text{Total Blank}})} \times \frac{100}{50} \times \frac{V_1}{V_2} \times \frac{f}{C_p}$$

$$\text{CuZn SOD activity (U/ml)} = i_{\text{CnZn SOD}} \times \frac{1}{50} \times \frac{V_1}{V_2} \times \frac{f}{C_p} = \frac{(\text{OD}_{\text{CuZn Control}} - \text{OD}_{\text{CuZn Sample}})}{(\text{OD}_{\text{CuZn Control}} - \text{OD}_{\text{CuZn Blank}})} \times \frac{100}{50} \times \frac{V_1}{V_2} \times \frac{f}{C_P}$$

$$\text{Mn activity (U/ml)} = \text{Total activity} - \text{CuZn activity}$$

Where:

$\text{OD}_{\text{T otal Control}}$	OD value of the Total SOD (Total) control
$\text{OD}_{\text{Total Sample}}$	OD value of the Total SOD (Total) sample
$\text{OD}_{\text{Total Blank}}$	OD value of the Total SOD (Total) blank
$\text{OD}_{\text{CuZn Control}}$	OD value of the CuZn SOD (CuZn) control
$\text{OD}_{\text{CuZn Sample}}$	OD value of the CuZn SOD (CuZn) sample
$\text{OD}_{\text{CuZn Blank}}$	OD value of the CuZn SOD (CuZn) blank
100	Conversion of inhibition ratio to percentage (100%)
50	Conversion of inhibition ratio to 50%
V_1	Total volume of the reaction system (ml)
V_2	Volume of sample added to the reaction system (ml)
C_P	Concentration of protein in sample (mg/ml)
f	The dilution factor of sample

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