(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Superoxide Dismutase (SOD) typed Assay Kit

Catalog No.: BC00174

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

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Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Superoxide Dismutase (SOD) typed Assay Kit
Detection Method	Colorimetric
Sample Type	Tissue, serum, plasma, cell, liquid samples
Assay Type	Quantitative
Detection Instrument	Spectrophotometer, Microplate reader (550 nm)

Product Introduction

Superoxide dismutase (SOD) plays a vital role in maintaining the balance between oxidation and antioxidation in organisms. This enzyme scavenges superoxide anion radicals ($O_2^{-}\cdot$) to protect cells from damage.

This kit adopts the xanthine oxidase method to detect the activity of Superoxide Dismutase (SOD). It can be used to measure SOD activity in serum (plasma), cerebrospinal fluid, pleural effusion, ascites, renal dialysate, urine, red blood cells, white blood cells, platelets, cultured cardiomyocytes, cultured tumor cells, various animal and plant tissue cells, as well as subcellular fractions (mitochondria, microsomes).

Principle

The xanthine-xanthine oxidase reaction system generates superoxide anion radicals ($O_2^{-}\cdot$). These radicals oxidize hydroxylamine to form nitrite, which develops a purplish-red color upon reaction with the chromogenic agent; the absorbance is measured using a visible light spectrophotometer. If the test sample contains SOD, the enzyme specifically inhibits superoxide anion radicals and reduces nitrite production. As a result, the absorbance of the test tube is lower than that of the control tube, and the SOD activity in the sample can be calculated via the corresponding formula.

There are only two types of SOD in higher animal cells: copper-zinc SOD (CuZn-SOD) and manganese SOD (Mn-SOD). The sum of their activities equals total SOD (T-SOD). After sample pretreatment, Mn-SOD activity is completely lost, while CuZn-SOD activity remains unchanged.

Components

No.	Components	Size	Notes
Reagent 1	Stock solution	10 mL	Partial crystallization may occur under low temperature or refrigeration. Heat to 37 °C to fully dissolve crystals before use.
Reagent 2	Liquid	10 mL	Store at 4 °C
Reagent 3	Liquid	10 mL	Store at 4 °C
Reagent 4	Stock solution	350 μL×2	Store below -20 °C. (For repeated use, aliquot and store after the first thawing.)
	Diluent	10 mL	Store at 4 °C
Reagent 5	Powder	1 vial	Store at 4 °C away from light after preparation.
Reagent 6	Powder	1 vial	Store at 4 °C away from light after preparation.
Reagent 7	Liquid	30 mL	Store at 4 °C
Consumable 1	Microplate	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Notes:

Preparation of Reagent 1 Working Solution: Dilute with double-distilled water at a ratio of 1:9 prior to use, and store at 4 °C.

Preparation of Reagent 4 Working Solution: Mix stock solution and diluent at a ratio of 1:14 before use. Prepare only the required volume and use immediately after preparation.

Preparation of Reagent 5 Working Solution: Add 75 mL double-distilled water before use (mark the liquid level). Heat the solution to 70-80 °C to dissolve the solute for later use. If water evaporates during heating and the liquid volume decreases, replenish with double-distilled water up to the marked line. The prepared reagent shall be stored at 4 °C protected from light.

Preparation of Reagent 6 Working Solution: Add 75 mL double-distilled water to dissolve before use, and store the prepared reagent at 4 °C protected from light.

Preparation of Chromogenic Agent: Mix Reagent 5 Working Solution, Reagent 6 Working Solution

and glacial acetic acid at a volume ratio of 3:3:2 to prepare the chromogenic agent. The prepared chromogenic agent can be stored at 4 °C protected from light for 3 months.

Storage

The shelf life of this kit is 6 months, and the storage temperature is 4 °C. Note that the stock solution of Reagent 4 must be stored below -20 °C; high temperature will easily inactivate the enzyme. All sampling tools should be clean, preferably sterilized or thoroughly rinsed repeatedly with double-distilled water. Contamination by bacteria and heavy metal ions must be strictly avoided.

Preparation

Spectrophotometer with a wavelength of 550 nm and 1 cm path-length cuvettes (or microplate reader and 96-well plates), 37 °C constant temperature water bath or dry incubator, bench-top low-speed centrifuge, pipettes of various specifications, double-distilled water, normal saline (0.9%) or 0.1 M PBS, glacial acetic acid (analytical grade, acetic acid concentration ≥99.5%), vortex mixer, disposable plastic test tubes (or centrifuge tubes), protein quantification reagents (for tissue and cell samples, available from our company), heating device (for reagent preparation heating).

- **Sample handling**

1. **Liquid Samples Such as Serum/Plasma:** Take an aliquot for preliminary testing (refer to Precautions). After determining the optimal concentration, proceed to Step 2 using the optimal concentration.

   Tissue samples: Accurately weigh the tissue. Add 9 volumes of homogenization medium (0.9% normal saline or 0.1 mol/L phosphate buffer solution with pH 7.0-7.4 is recommended) at a mass-to-volume ratio (g:mL) of 1:9. Homogenize mechanically in an ice-water bath, then centrifuge at 4000 rpm for 10 minutes and collect the supernatant (10% tissue homogenate supernatant). Determine the optimal concentration via preliminary testing, then carry out Step 2 with the optimal concentration.

   Cell samples: Harvested cells are washed 1 to 2 times with PBS, followed by low-speed centrifugation to collect cell pellets. Resuspend the pellets in 0.3-0.5 mL isotonic 0.1 M PBS buffer (pH 7.4), then lyse the cells by sonication or manual grinding. If no obvious solid precipitates remain after lysis, the lysate can be used directly after preliminary testing to

determine the optimal sampling concentration. If significant solid residues are observed, centrifuge the lysate at low speed (2000 rpm) for 5-10 minutes, collect the supernatant for preliminary testing, and then proceed to Step 2. (It is recommended that each sample contain more than 10^6 cells.)

2. Take 0.2 mL of the above sample and add 0.2 mL of Reagent 7. Mix thoroughly with a vortex mixer for 1 minute, then centrifuge at 3500-4000 rpm for 15 minutes. Collect the supernatant for CuZn-SOD detection (this processed supernatant loses Mn-SOD activity while CuZn-SOD activity remains intact).
   Meanwhile, take 0.2 mL of normal saline and add 0.2 mL of Reagent 7. Mix thoroughly with a vortex mixer for 1 minute, centrifuge at 3500–4000 rpm for 15 minutes, and collect the supernatant as the CuZn-SOD control.

Operation process

	T-SOD Control	T-SOD Assay	CuZn-SOD Control	CuZn-SOD Assay
Reagent 1 Working Solution (mL)	1.0	1.0	1.0	1.0
Double-distilled water (mL)	a			
Step 1 sample supernatant (mL)		a		
Step 2 control supernatant (mL)			a	
Step 2 detection supernatant (mL)				a
Reagent 2 (mL)	0.1	0.1	0.1	0.1
Reagent 3 (mL)	0.1	0.1	0.1	0.1
Reagent 4 Working Solution (mL)	0.1	0.1	0.1	0.1
Mix thoroughly using a vortex mixer, then incubate in a 37°C constant-temperature water bath for 40 minutes.				
Chromogenic Agent (mL)	2.0	2.0	2.0	2.0
Mix well and allow to stand at room temperature for 10 minutes.				

Set the wavelength to 550 nm with a 1 cm optical path length, zero the spectrophotometer with double-distilled water, and measure the absorbance of each tube. Alternatively, transfer 0.2 mL reaction mixture into a 96-well plate and read the absorbance at 550 nm using a microplate reader. For calculations based on 96-well plate data, the blank well absorbance of each well must be

subtracted individually.

Note: a refers to the volume of sample and double-distilled water added.

Calculation

1. Calculation of Total SOD and CuZn-SOD Activity in Liquid Samples Such as Serum and Plasma:

   Definition: One unit (U) of SOD activity is defined as the amount of SOD required to achieve a 50% inhibition rate in the reaction mixture per milliliter of sample.

   Calculation formula:

$$\text{SOD Activity (U/mL)} = \frac{A_{\mathbf{Control}} - A_{\mathbf{Assay}}}{A_{\mathbf{Control}}} \div 50\% \times \frac{V_{\text{Total}}}{V_{\text{Sample}}} \times N$$

2. Calculation of Total SOD and CuZn-SOD Activity in Tissues Samples:

   Definition: One unit (U) of SOD activity is defined as the amount of SOD that achieves a 50% inhibition rate in 1 mL reaction mixture per milligram of tissue protein.

   Calculation formula:

$$\text{SOD Activity (U/mg)} = \frac{A_{\mathbf{Control}} - A_{\mathbf{Assay}}}{A_{\mathbf{Control}}} \div 50\% \times \frac{V_{\text{Total}}}{V_{\text{Sample}}} \div \text{Cpr}$$

Annotation:

V_{Total}: Total volume of reaction solution, (a + 3.3) mL

V_{Sample}: Sample volume, a mL

N: Dilution factor of the sample before testing

Cpr: Protein concentration at the corresponding tissue sample concentration, mg/mL

Precautions

1. Wear a lab coat and disposable gloves during operation.
2. This product is intended for scientific research use only by professionals and must not be used

for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.

3. The optimal sample volume varies with sample types due to differing SOD activity levels. Since the percentage inhibition rate of the enzyme shows a parabolic correlation with enzyme activity, different samples require distinct sampling volumes. It is recommended to determine the optimal sample volume before testing each new type of sample.
4. Determination of Optimal Sample Volume: When testing a new type of sample with this kit for the first time, it is recommended to prepare three test tubes with different sample volumes in advance. Take 10 μL, 30 μL and 50 μL sample separately to prepare three sample tubes and one control tube, then conduct a preliminary test following the Total-SOD operating protocol to confirm the optimal sample volume.
5. Calculation of Inhibition Rate: Inhibition rate = $(A_{Control} - A_{Assay}) \div A_{Control}$
6. Optimal Inhibition Range: The inhibition rate shall be kept between 0.15 and 0.55 (the curve within this range presents an approximately linear correlation).
7. Selection of Optimal Sample Volume: Adopt the sample volume of the tube with an inhibition rate around 45%-50% as the optimal volume.
8. Adjustment of Sample Volume: If the inhibition rate exceeds 60% (the flat segment of the curve), dilute the sample or reduce the sample volume and retest. If the inhibition rate is lower than 20%, increase the sample volume for retesting.
9. Add reagents in accordance with the sequence listed in the operation table; do not prepare mixed reagents in advance.
10. This practice greatly facilitates the analysis of research results and t-test analysis. If the percentage inhibition rate is higher than 60% or lower than 10%, the results of each test group often show no significant difference in t-tests.
11. The protein concentration of tissue homogenate shall be measured simultaneously.

Reference Values for Optimal Sample Volume

Red blood cell extract: 8.0-10 μL

Rat red blood cell extract: 5 μL

Human plasma (serum): 30-50 μL

Mouse plasma (serum): 20 μL

1% tissue homogenate: 30-50 μL

Cytoplasm: 20-50 μL

Myocardial perfusate or renal dialysate: 100-200 μL

Leukocyte suspension: 100-200 μL

Cell culture medium: 100-200 μL