# SUPEROXIDE DISMUTASE

**Colorimetric Method** **50 Tests**

**For Research Only**

## INTRODUCTION :

Superoxide dismutases (SODs) are metalloenzymes that catalyze the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide and thus form a crucial part of the cellular antioxidant defense mechanism.

$2O_2^{\cdot} + 2H^+ + SOD \longrightarrow H_2O_2 + O_2$

Three types of SODs have been characterized according to their metal content: copper zinc (Cu/Zn), manganese (Mn), and iron (Fe). SOD is widely distributed in both plants and animals. It occurs in high concentrations in brain, liver, heart, erythrocytes, and kidney. In humans there are three forms of SOD: cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD. Extracellular SOD is found in the interstitial spaces of tissues and also in extracellular, accounting for the majority of the SOD activity in plasma, lymph, and synovial fluid.

The amount of SOD present in cellular and extracellular environments is crucial for the prevention of diseases linked to oxidative stress.

## PRINCIPLE :

This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye .

## REAGENTS :

| | | |
|---|---|---|
| **1.** | Phosphate Buffer pH 8.5 | 50 mM/L |
| **2.** | Nitroblue tetrazolium (NBT) | 1 mM/L |
| **3.** | NADH | 1 mM/L |
| **4.** | Phenazine methosulphate (PMS) | 0.1 mM/L |
| **5-** | Extraction Reagent | |

## PREPARATION OF SOLUTION:

- Reagent 1, ready for use.
- Reagent 2, reconstitute in 5 ml d. Water.
- Reagent 3, reconstitute in 5 ml buffer.
- Reagent 4, reconstitute in 5 ml d. Water, **Dilute 1000 times immediatly before use (10 µL +10 ml d. Water) discard after use.**

## STABILITY:

The reagents are stable up to the expiry date specified when stored at the proper temperature indicated

- R1 Store at 2 -8˚C.
- R2, R3, R4 Store at - 20˚C or below.
- R3 after reconstitution stable for 24 hrs in closed container at -4˚C or below so most samples should be prepared before assay.

## CONTROL OF THE PROCEDURE :

This control can be performed by measuring the SOD activity of a mammalian Cu/Zn-SOD solution. This solution may be the supernatant of an erthrocyte lysate or a purified erthrocyte enzyme solution. Aliquots such an enzyme solution should be made and stored at -70 °C to serve as controls. The SOD activity remains stable for at least 6 months under the above conditions. The assay must be performed on an aliquot thawed immediately prior to the measurement.

## PROCEDURE : (See Sample Preparation)

- **R4 should be diluted 1000 times immediately before use (10 µL + 10 ml dist.water), discard after use.**
- **Sample should be diluted to give an inhibition percent between 30 and 60.**
- **Working Reagent : Mix R1 + R2 + R3 in ratio of (10+1+1 ml ), Immediatly before use.**

| | Control ml | Sample ml |
|---|---|---|
| **Working Reagent** | **1.0** | **1.0** |
| **Sample** | **-** | **0.1** |
| **D. Water** | **0.1** | **-** |
| | **Mix well. Initiate the reaction by the addition of :** | |
| **PMS ( R4 )** | **0.1** | **0.1** |

Measure the increase in absorbance at 560 nm for 5 min for control ($\triangle A_{control}$ ) and for sample ( $\triangle A_{sample}$) at 25˚C.

## CALCULATION :

$$\text{Percent inhibition} = \frac{\triangle A_{control} - \triangle A_{sample}}{\triangle A_{control}} \times 100$$

Where

$\triangle A_{control}$ =

The change in absorbance at 560 nm over 5 min. following the addition of PMS to the reaction mixture in the absence of sample .

$\triangle A_{sample}$ =

The change in absorbance at 560 nm over 5 min. following the addition of PMS to the reaction mixture in the presence of sample .

Purified SOD was shown to inhibit the initial rate of photo activated phenazine methosulfate mediated reduction of $O_2^{\cdot}$ to $O_2$ which then reduced nitroblue tetrazolium. 1.5 U/assay of the purified enzyme produced 80% inhibition.

SOD activity can be expressed as a function of any other relevant parameter such as protein or hemoglobin content which has been measured separately.

## SOD Activity:

**U/ml = % inhibition x 3.75**

$$\text{U/gm tissue} = \text{\% inhibition} \times 3.75 \times \frac{1}{\text{gm tissue used}}$$

$$\text{U/gm Hb} = \text{\% inhibition} \times 3.75 \times \frac{1}{\text{gm Hb used}}$$

## SAMPLE PREPARATION

**All samples should be prepared befor reconstitution of the reagents.**

### Erythrocyte lysate

1. Use heparinized or EDTA whole blood samples.it is recommended that erythrocytes should be washed four times with 0.9 %NaCl solution.
2. Centriifuge 0.5 ml of whole blood for 10 minutes at 4000 rpm and then aspirate Off the plasma.
3. Then wash erythrocytes four times with 3 ml of 0.9 % NaCl solution centrifuging for 10 minutes at 4000 rpm after each wash .
4. The washed centrifuged erythrocytes should then be made up to 2.0 ml with cold redistilled water , mixed and left to stand at +4 °C for 15 minutes . if not assayed immediately store at -70 .The lysate is diluted with d. water , so that the % inhibition falls between 30 % and 60 % .

**5**. A 25 fold dilution of lysate is recommended for human samples ( final dilution factor = 100 ) and a 50 fold dilution for bovine samples ( final dilution factor = 200 )

### Whole tissue:

**1**-Prior to dissection, perfuse tissue with a PBS (phosphate buffered saline ) solution, pH 7.4 . containing 0.16 mg / ml heparin to remove any red blood cells .

**2**. Homogenize the tissue in 5 – 10 ml cold buffer ( i , e , 100 mM potassium phosphate, pH 7.0, containing 2 mM EDTA ) per gram tissue.

**3**. Centrifuge at 4,000 rpm for 15 minutes at 4 °C.

**4**- Collect supernatant, If not assayed Immediately store the supernatant at -20°C.

**5-** Add 0.5 mL of Ice-cold extraction reagent to 1.0 ml of supernatant in glass test tube.

**6**- Vortex for at least 30 seconds.

**7**- Centrifuge at 4000 rpm and 4 °C for 10 minutes.

**8-** Collect the aqueous upper layer and keep at 0-4 °C for immediat assay.

### Elimination of Interference in Tissue Sample:

- The SOD assays subject to interferences which are mainly due to the presence of one or several compounds exhibiting redox properties, and most often a mercaptan RSH, in the sample.
- Add 0.5 mL of ice-coled absolute ethanol/chloroform 60/40 (v/v) to 1.0 mL sample in glass test tube. Thoroughly mix for at least 30 seconds and centrifuge at 4°C and 4000 rpm for 10 minutes. The resulting supernatant should be stored at 0-4°C untill used for the assay.

## WARNINGS AND PRECAUTIONS

- For in vitro use only.
- Do not smoke, eat or drink in areas where samples and reagents are manipulated.
- Wear disposable gloves during manipulation of reagents and samples.
- Mouth pipetting is strictly prohibited. In case of accidental exposure of skin, mucous membranes or eyes, to R2 or R4 reagents, thoroughly wash the exposed area with water.

## REFERENCE:

Nishikimi, M., Roa, N.A., and Yogi, K ( 1972 )
Biochem. Bioph. Res. Common., 46, 849 – 854

BIODIAGNOSTIC
DIAGNOSTIC AND RESEARCH REAGENTS

# SUPEROXIDE DISMUTASE

| | |
|---|---|
| **R1 at 4˚C**<br>**R2, R3, R4 at -20˚C** | **50 Tests** |
| **CAT. No.** | **SD 25 21** |

**FOR RESEARCH USE ONLY**

## REAGENTS

| | | | |
|---|---|---|---|
| **R1** | **Buffer** | **60** | **ml** |
| **R2** | **NBT** | **1** | **vial** |
| **R3** | **NADH** | **1** | **vial** |
| **R4** | **PMS** | **1** | **vial** |
| **R5** | **Extraction Reagent** | **25** | **ml** |

## CONTACTS

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