

SUPEROXIDE DISMUTASE

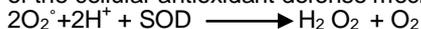
Colorimetric Method

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.



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 ($\Delta A_{\text{control}}$) and for sample (ΔA_{sample}) at 25°C.

CALCULATION :

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

Where

$$\Delta A_{\text{control}} =$$

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

$$\Delta A_{\text{sample}} =$$

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:

$$U/ml = \% \text{ inhibition} \quad \times 3.75$$

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

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

