Super Oxide Dismutase (SOD)

Cat.No: Otto3047

Summary & Explanation

Superoxide dismutase (SOD) is an important antioxidant enzyme that plays a key role in the body's defense against oxidative stress. It catalyzes the conversion of the highly reactive superoxide anion ($O_2^{\bullet -}$) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), thereby reducing cellular damage caused by reactive oxygen species (ROS).

Test Principle

Superoxide dismutase (SOD) catalyzes the dismutation of superoxide anion radicals ($O_2^{\bullet -}$) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). In this colorimetric assay, superoxide radicals generated by the reaction system react with a chromogenic reagent to produce a colored compound.

SOD present in the sample competes with the chromogenic reaction by removing superoxide radicals, thereby inhibiting the formation of the colored product. Therefore, the degree of inhibition is directly related to the SOD activity in the sample.

SOD activity can be determined by measuring the optical density (OD) at the specified wavelength and calculating the inhibition of color formation relative to the control.

Kit Companents

Content	Component	Size
Reagent 1	Buffer solution	30ml
Reagent 2	Chromogen solution	15ml
Reagent 3	Generator solution	15ml

Reagent and Sample Preparation

Sample Dilution (1:50): Dilute serum 1:50 with purified/deionized water before analysis. For example, add 200 µL serum to 9.8 mL purified water to obtain a final volume of 10.0 mL.

R3 Working Reagent: Prepare the R3 working reagent immediately before use by adding 40 µL R3 concentrate to 10.0 mL purified/deionized water and mixing thoroughly.

Storage & Stability

Reagent: Stable up to expiry date when stored capped and at +4°C even after start using

Reactivity

This kit can be used for detection of SOD in serum, plasma, salive, animal and plant tissue samples.

Specimen

Serum: Collect serum using regular sampling tubes. Let the samples clot for 1 hour at room temperature, maintaining a temperature of 2-8°C. Afterward, centrifuge the samples for 10 minutes at 3000×g at 2-8°C. Retrieve the supernatant to perform the assay.

Plasma: Collect plasma using heparin as an anticoagulant. Centrifuge the samples for 10 minutes at 3000×g at 2-8°C, ensuring this is done within 30 minutes of collection.

Tissue homogenates: For general reference, hemolyzed blood may influence the results. Therefore, tissues should be cut into small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) or KCl (140 mM) to thoroughly remove any excess blood. The tissue pieces should be weighed and then homogenized in PBS using a glass homogenizer on ice, with a ratio of tissue weight (g) to PBS volume (mL) of 1:9. The homogenate is then centrifuged for 5 minutes at 7000×g at 2-8°C to obtain the supernatant.

Assay Range

2 - 500 U/ml

Refference Range

Each laboratory is recommended to establish their own reference values.

Assay Procedure

Step	Component / Procedure	Volume / Condition
1	Add R1 reagent	150 µL
2	Add diluted sample (S)	15 µL
3	Add R2 reagent	15 µL
4	Add prepared R3 working reagent	15 µL
5	Mix all components thoroughly	Mix well
7	Measure absorbance	15 minutes
8	Measure absorbance	30 minutes
9	Measurement wavelength	560 nm

Water Blank / Control: Run purified/deionized water as the blank/control. Perform five replicate measurements and use the mean absorbance value for the calculation.

Calculation

SOD activity (U/mL) = [(Delta Water Abs - Delta Sample Abs) / (Delta Water Abs/2)] X 650

Warning

For in vitro use only
Do not pipette by mouth
Do not use reagents beyond the expiry date.

Important Notes

*Use the same incubation time for blank/control and sample measurements.
*All reagents should be mixed thoroughly without introducing excessive bubbles before absorbance measurement.
*The calculation above is based on the stated 1:50 sample dilution and assay factor. If the sample dilution is changed, the final calculation factor must be adjusted accordingly.
*For routine use, the selected reading time should be established and fixed during assay validation.

References

1. Kono Y. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Arch Biochem Biophys. 1978;186(1):189-195. doi:10.1016/0003-9861(78)90479-4.

2. Beauchamp C, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal Biochem. 1971;44(1):276-287. doi:10.1016/0003-2697(71)90370-8.

3. Elstner EF, Heupel A. Inhibition of nitrite formation from hydroxylammoniumchloride: A simple assay for superoxide dismutase. Anal Biochem. 1976;70(2):616-620. doi:10.1016/0003-2697(76)90488-7.

4. Beyer WF Jr, Fridovich I. Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. Anal Biochem. 1987;161(2):559-566. doi:10.1016/0003-2697(87)90489-1.

5. Oyanagui Y. Reevaluation of assay methods and establishment of kit for superoxide dismutase activity. Anal Biochem. 1984;142(2):290-296. doi:10.1016/0003-2697(84)90467-6.

6. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem. 1988;34(3):497-500. doi:10.1093/clinchem/34.3.497.

7. Nishikimi M, Appaji Rao N, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun. 1972;46(2):849-854. doi:10.1016/S0006-291X(72)80218-3.