MA-0108

Superoxide Dismutase Activity Assay

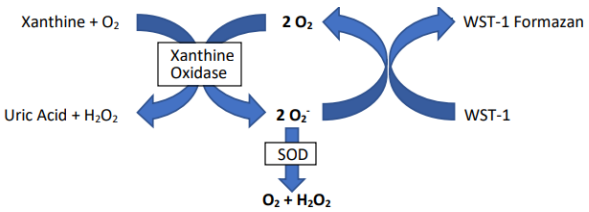
(100 wells, Absorbance-based, OD 450 nm, Store at 4°C)

Background Information:

Superoxide, the one electron reduced form of dioxygen is formed as a side product of several redox enzymes, such as mitochondrial complex I and complex II during respiration and NADPH oxidase as a part of the immune system. Superoxide is quite toxic due to its high reactivity and the level of superoxide must be controlled in living organisms. Superoxide dismutase (SOD) is the enzyme which fulfills this role. SOD catalyzes the dismutation of superoxide anions into hydrogen peroxide and molecular oxygen, both much less toxic chemicals. In AkrivisBio's SOD Activity Assay, superoxide formed by xanthine oxidase reduces WST from a nearly colorless tetrazolium to a highly colored formazan. In the presence of SOD, superoxide is scavenged, in an amount proportional to the SOD activity present and color development is reduced accordingly.

Assay Principle:

1 – Xanthine Oxidase forms superoxide during oxidation of xanthine
2 – Superoxide reduces WST-1 to its formazan
3 – In the presence of SOD, superoxide is destroyed preventing color formation



Assay Components:

Assay Buffer	20 ml	WM	MA-0108A
Dilution Buffer	10 ml	NM	MA-0108B
Xanthine Oxidase	20 µl	Green	MA-0108C
WST Solution	1 ml	Red	MA-0108D
Superoxide Dismutase Positive Control	50 µl	Blue	MA-0108E

User Supplied Equipment:

Multichannel pipettor

Storage and Handling: Store kit at 4°C

WST Solution: Dilute 1 ml of WST solution with 19 ml of SOD Assay Buffer Solution. The diluted solution is stable for up to 2 months.
Xanthine Oxidase: Reconstitute with 2 ml of dilution buffer. The reconstituted Xanthine Oxidase is stable for up to 3 weeks.
Superoxide Dismutase: Dissolve in 50 µl Dilution Buffer to make 2 U/µl

Sample Preparation:

Inhibition Curve using Positive Control:

Take 5 µl of SOD	+	995 µl Dilution Buffer =	10 U/ml	(A)
Take 100 µl A	+	100 µl Dilution Buffer =	5 U/ml	(B)
Take 100 µl B	+	100 µl Dilution Buffer =	2.5 U/ml	(C)
Take 100 µl C	+	100 µl Dilution buffer =	1.25 U/ml	(D)
Take 100 µl D	+	100 µl Dilution Buffer =	0.625 U/ml	(E)
Take 100 µl E	+	100 µl Dilution Buffer =	0.313 U/ml	(F)

Samples:

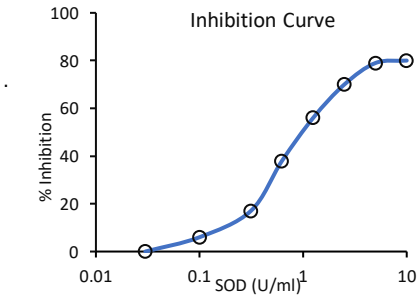
1. **Blood:** Collect blood using anticoagulant. Centrifuge at 1,000 x g for 10 min at 4°C. Transfer the plasma to a fresh tube avoiding the buffy layer and store at -80°C.Aspirate away the buffy layer from the red cell pellet. Resuspend the red cells in 5X volume of ice-cold distilled water. Centrifuge at 10,000 X g for 10 min to pellet the erythrocyte membranes. Remove supernatant for analysis. Store at -80°C.
   For analysis, dilute plasma 3 - 10X.Dilute red cell lysate 100X.
2. **Tissue/ cells:** Perfuse tissue with 150 mM KCl to remove red blood cells. Homogenize tissue or cells in ice-cold 0.1 M Tris/HCl, pH 7.4 with 0.5% Tergitol, 5 mM β-ME, 0.1 mg/ml PMSF. Centrifuge the crude homogenate/lysate at 14,000 X g for 5 minutes and discard the cell debris. The supernatant contains total SOD activity from cytosolic and mitochondria and can be sub fractionated if desired.

SOD Assay Protocol:

Refer to Table below for reagent volumes to be used for all samples and Positive Controls. Each sample requires 4 separate wells.

1. Add 20 µl of Sample to each sample and blank 2 well and add 20 µl H_2O to each Blank 1 and Blank 3 well.
2. Add 200 µl of diluted WST Solution to each well.
3. Add 20 µl of Dilution Buffer to each Blank 2 and Blank 3 well.
4. Add 20 µl of Xanthine Oxidase solution to each sample and Blank 1 well as quickly as possible, using a multichannel pipettor.
5. Incubate plates at 37°C for 20 min.
6. Read the absorbance at 450 nm using a microplate reader.
7. Calculate the SOD activity (inhibition rate %) using the following equation.

$$\text{SOD Activity (inhibition rate \%)} = \frac{[(Blank1 - Blank3) - (Sample - Blank2)]}{(Blank1 - Blank3)} \times 100$$



	sample	blank 1	blank 2	blank 3
Sample Solution	20 µl		20 µl	
ddH_2O		20 µl		20 µl
WST Working Solution	200 µl	200 µl	200 µl	200 µl
Enzyme Working Solution	20 µl	20 µl		
Dilution Buffer			20 µl	20 µl

Unit Definition: One Unit is defined as the amount of SOD that inhibits the XO activity by 50% IC50 in µg, under the assay conditions.

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