Superoxide Dismutase Activity Assay Kit

Catalog Number KA0783

100 assays

Version: 03

Intended for research use only

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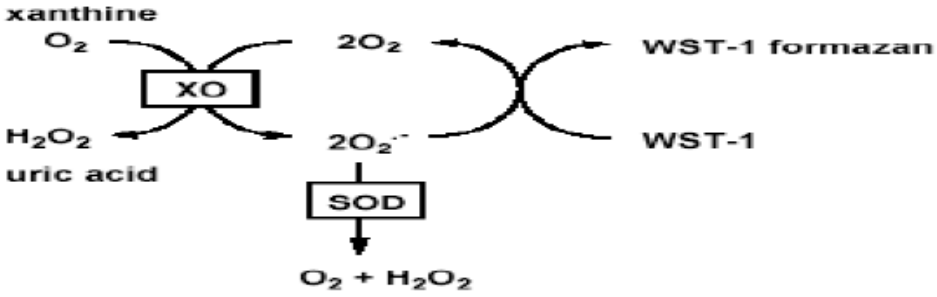
Introduction

Background

Superoxide dismutase (SOD) is one of the most important antioxidative enzymes. It catalyzes the dismutation of the superoxide anion into hydrogen peroxide and molecular oxygen. The Superoxide Dismutase (SOD) Activity Assay Kit utilizes WST-1 that produces a water-soluble formazan dye upon reduction with superoxide anion. The rate of the reduction with a superoxide anion is linearly related to the xanthine oxidase (XO) activity, and is inhibited by SOD. Therefore, the inhibition activity of SOD can be determined by a colorimetric method.



General Information

Materials Supplied

List of component

Component	Amount
WST Solution	1 ml
SOD Enzyme Solution	20 µl
SOD Assay Buffer	20 ml
SOD Dilution Buffer	10 ml

Storage Instruction

Store kit at 4^{o}C for one year

Assay Protocol

Reagent Preparation

- WST Working Solution: Dilute the 1 ml of WST solution with 19 ml of Assay Buffer Solution. The diluted solution is stable for up to 2 months at 4℃.
- Enzyme Working Solution: Centrifuge the Enzyme Solution for 5 seconds. Mix well by pipeting (The step is necessary, as the enzyme has two layers and must be mixed well before dilution). Dilute 15 µl with 2.5 ml of Dilution Buffer. The diluted enzyme solution is stable for up to 3 weeks at 4℃.

Sample Preparation

- Blood samples: Collect blood using citrate or EDTA. Centrifuge at 1,000 x g for 10 min at 4℃. Transfer the plasma layer to a new tube without disturbing the buffy layer and store at -80℃ until ready for analysis. Remove the buffy layer from the red cell pellet. Resuspend the erythrocytes in 5x volume of ice cold distilled water and centrifuge at 10,000 x g for 10 min to pellet the erythrocyte membranes. Store the supernatant at -80℃ until ready for analysis. Plasma can be diluted approx. 3-10x and the red cell lysate diluted approx. 100x prior to SOD assay.
- Tissue and cells: Tissue should be perfused with PBS or 150mM KCl to remove any red blood cells. Homogenize tissue or lyse cells in ice cold 0.1M Tris/HCl, pH 7.4 containing 0.5 % Triton X-100, 5mM β-ME, 0.1mg/ml PMSF. Centrifuge the crude tissue homogenate/cell lysate at 14000 x g for 5 minutes at 4℃ and discard the cell debris. The supernatant contains total SOD activity from cytosolic and mitochondria.

Assay Procedure

Refer to Table 1 for the amount of solution in each well. If you are using a SOD standard (not included with the kit), set up wells for it in the same manner as the sample.

Table 1: Amount of each solution for sample, blank1, 2, and 3

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

1. Add 20 µl of Sample Solution to each sample and blank 2 well and add 20 µl H_2O to each Blank 1 and Blank 3 well (See Table 1).
2. Add 200 µl of the WST Working Solution to each well.

3. Add 20 µl of Dilution Buffer to each Blank 2 and Blank 3 well.
4. Add 20 µl of Enzyme Working solution to each sample and Blank 1 well, mix thoroughly.
   Note: since the superoxide will release immediately after the addition of Enzyme working Solution to each well, use a multiple channel pipette to avoid reaction time lag of each well.
5. Incubate plates at 37℃ for 20 minutes.
6. Read the absorbance at 450 nm using a microplate reader.

Data Analysis

Calculation of Results

The SOD activity (inhibition rate%) using the following equation.

$$\text{SOD Activity (inhibition rate\%)} = \frac{(A_{blank1} - A_{blank3}) - (A_{sample} - A_{blank2})}{(A_{blank1} - A_{blank3})} \times 100$$