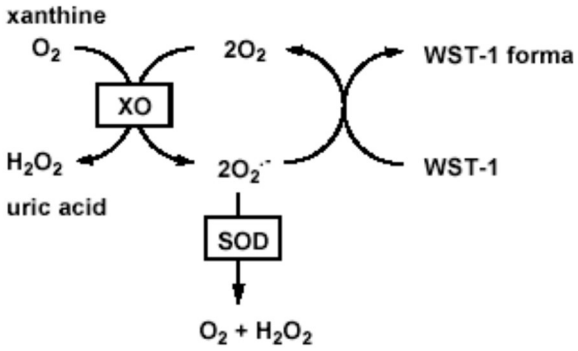
Technical Bulletin

Superoxide Dismutase (SOD) Activity Kit

Catalog Number MAK379

Product Description

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 sensitive SOD 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 (below). Therefore, the inhibition activity of SOD can be determined by a colorimetric method.



The kit is suitable for the determination of superoxide dismutase (SOD) activity in blood, tissue and cell lysates.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- WST Solution 1 mL
  Catalog Number MAK379A
- SOD Enzyme Solution 20 µL
  Catalog Number MAK379B
- SOD Assay Buffer 20 mL
  Catalog Number MAK379C
- SOD Dilution Buffer 10 mL
  Catalog Number MAK379D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (including multichannel pipettor)
- 96-well flat-bottom plate. It is recommended to use clear plates for colorimetric assays. Cell culture or tissue culture treated plates are **not** recommended.
- Spectrophotometric multiwell plate reader
- Refrigerated microcentrifuge capable of RCF ≥14,000 × *g*
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)

- Mitochondria/Cytosol Fractionation Kit (Catalog Number MIT1000)
- Phosphate Buffered Saline (Catalog Number P3813 or equivalent)
- Trizma® hydrochloride (Catalog Number T3253 or equivalent)
- Triton™ X-100 (Catalog Number X100)
- 2-Mercaptoethanol (Catalog Number M6250 or equivalent)
- Phenylmethanesulfonyl fluoride (PMSF) (Catalog Number P7626)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at 2-8 °C. Briefly centrifuge small vials at low speed prior to opening.

Preparation Instructions

WST Working Solution: Dilute the 1 mL of WST solution with 19 mL of SOD Assay Buffer. The diluted solution is stable for up to 2 months at 2-8 °C.

Enzyme Working Solution: Centrifuge the SOD Enzyme Solution for 5 seconds. Mix well by pipetting (The step is necessary, as the enzyme has two layers and must be mixed well before dilution). Dilute 15 µL of the mixed enzyme with 2.5 mL of SOD Dilution Buffer. The diluted enzyme solution is stable for up to 3 weeks at 2-8 °C.

Sample Preparation

Blood samples: Collect blood using citrate or EDTA. Centrifuge at 1,000 × g for 10 minutes at 2-8 °C. Transfer the plasma layer to a new tube without disturbing the buffy layer and store at -80 °C until ready for analysis. Remove the buffy layer from the red cell pellet. Resuspend the erythrocytes in 5× volume of ice-cold purified water and centrifuge at 10,000 × g for 10 minutes to pellet the erythrocyte membranes. Store the supernatant at -80 °C until ready for analysis. Plasma can be diluted approximately 3 – 10× and the red cell lysate diluted approximately 100× prior to SOD assay.

Tissue and cells: Tissue should be perfused with PBS to remove any red blood cells. Homogenize tissue or lyse cells in ice-cold 0.1 M Tris-HCl, pH 7.4 containing 0.5% Triton X-100, 5 mM 2-mercaptoethanol, 0.1 mg/ml PMSF. Centrifuge the crude tissue homogenate/cell lysate at 14000 × g for 5 minutes at 2-8 °C and discard the cell debris. The supernatant contains total SOD activity from cytosolic and mitochondria.

Procedure

Note: If it is desired to measure SOD activity from cytosol and mitochondria separately, cytosol and mitochondria can be separated by using the Mitochondria/Cytosol Fractionation Kit. SOD activity is then measured from the Mitochondria and Cytosol fractions separately.

Assay Procedure

1. Refer to Table 1 for the preparation of sample(s) and blank reaction mixes. Note: A SOD standard is **not** included with the kit. If desired, set up wells for standards in the same manner as the sample. Since the superoxide will release immediately after the addition of Enzyme Working Solution to each well, use a multichannel pipettor to avoid reaction time lag of each well.

Table 1. Reaction Mix Preparation

Reagent	Sample (s)	Blank 1	Blank 2	Blank 3
Sample(s)	20 µL		20 µL	
Purified Water		20 µL		20 µL
WST Working Soln.	200 µL	200 µL	200 µL	200 µL
Enzyme Working Soln.	20 µL	20 µL		
Dilution Buffer			20 µL	20 µL

2. Mix well and incubate plates at 37 °C for 20 minutes.

Measurement

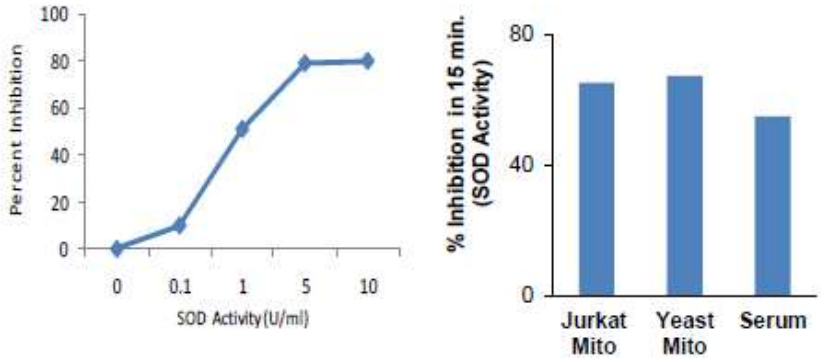
1. Read the absorbance at 450 nm using a microplate reader.
2. Calculate the SOD activity (inhibition rate %):

Results

SOD Activity (inhibition rate %) =

$$\frac{(A_{450}\,\text{blank1} - A_{450}\,\text{blank3}) - (A_{450}\,\text{sample} - A_{450}\,\text{blank2})}{(A_{450}\,\text{blank1} - A_{450}\,\text{blank3})} \times 100$$

Figure 1.



SOD Activity (% inhibition rate): human serum (10 µL) and isolated mitochondria from Jurkat cells (10 µg), and yeast (*Saccromyces cerevisiae, 100* µg), was used to determine SOD Activity according to the kit protocol. Activity was measured at 15 minutes at 37 °C.

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