SOD Activity Assay

KB-03-011

100/200/400 test (96 well plate)

BQCkit

A brand of Bio QuChem

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All chemicals should be handled with care

- This kit is for R&D use only

Introduction

Superoxide dismutases (SODs) are metallo enzymes that catalyse the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide and thus form a crucial part of the cellular antioxidant defense mechanism.

Excessive reactive oxygen species, especially superoxide anion ($O_2^{\bullet-}$), play important roles in the pathogenesis of many cardiovascular diseases, including hypertension and atherosclerosis. Superoxide dismutases (SODs) are the major antioxidant defense systems against $O_2^{\bullet-}$, which consist of three isoforms of SOD in mammals: the cytoplasmic Cu/ ZnSOD (SOD1), the mitochondrial MnSOD (SOD2), and the extracellular Cu/ZnSOD (SOD3), all of which require catalytic metal (Cu or Mn) for their activation.

Superoxide Dismutase Activity Assay Kit (Colorimetric) is a sensitive kit using 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.

Materials

BQCkit SOD Activity Assay kit *KB03011-100 tests* contains:

Product	Quantity	Storage
Reagent A	2 mL	4ºC
Reagent B	20 µL	4ºC
Reagent C	40 mL	4ºC
Reagent D	10 mL	4ºC
SOD Standard (3U/mL)	50 µL	4ºC

BQCkit SOD Activity Assay kit *KB03011-200 tests* contains:

Product	Quantity	Storage
Reagent A	4 mL	4ºC
Reagent B	40 µL	4ºC
Reagent C	80 mL	4ºC
Reagent D	20 mL	4ºC
SOD Standard (3U/mL)	100 µL	4ºC

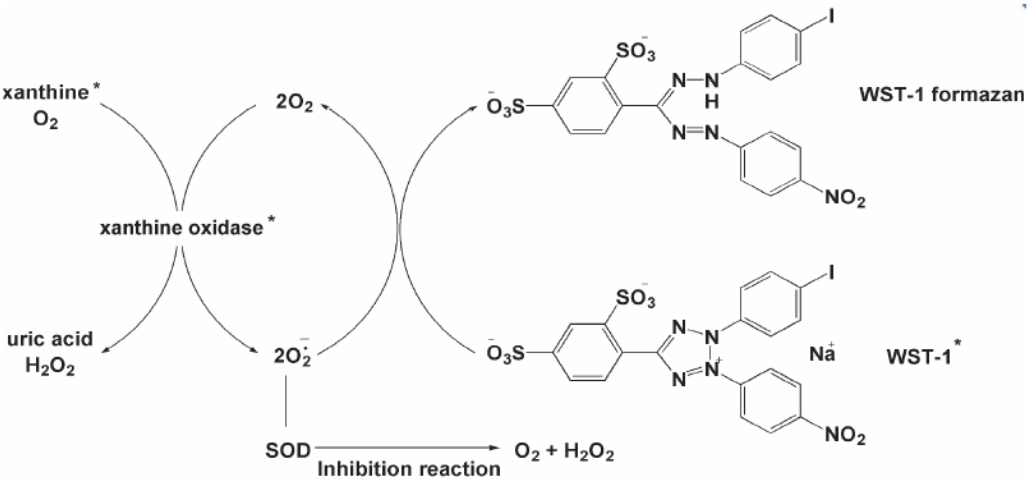
Materials

BQCkit SOD Activity Assay kit *KB03011-400 tests* contains:

Product	Quantity	Storage
Reagent A	8 mL	4ºC
Reagent B	80 µL	4ºC
Reagent C	160 mL	4ºC
Reagent D	40 mL	4ºC
SOD Standard (3U/mL)	200 µL	4ºC

Assay Principle

Bioquochem Superoxide Dismutase Activity Assay kit utilizes a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine (Scheme 1). One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical.



Scheme 1. *SOD inhibition assay mechanism*

XOD and SOD Antagonism in the Generation of Formazan Dye. The conversion of xanthine and O_2 to uric acid and H_2O_2 by XOD generates superoxide radicals. The superoxide anions reduce a tetrazolium salt (WST-1) to a colored formazan product (WST-1 formazan) that absorbs light. SOD scavenges superoxide anions, thereby reducing the rate of formazan dye formation.

Reagent Preparation

For 100 assays*:

*For a number of assays different from 100, recalculate Reagents volumes.

Working Reagent:

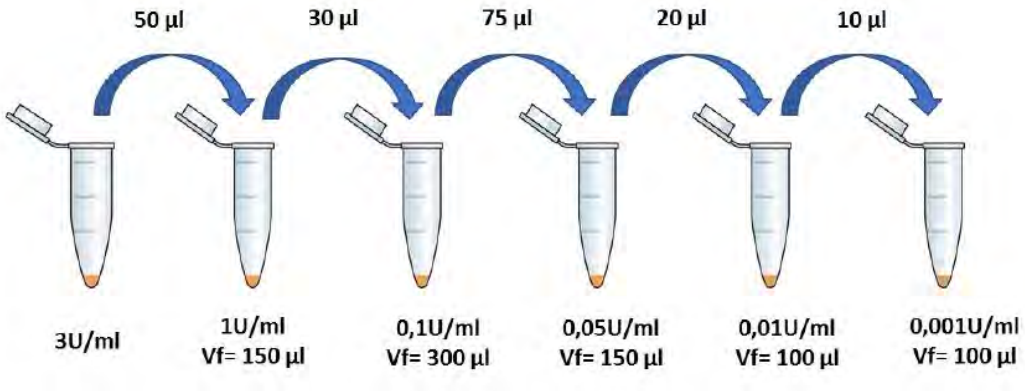
Mix 2 mL of Reagent A with 38 mL of Reagent C.

Enzyme Solution:

Centrifuge Reagent B in a microcentrifuge, and then mix by pipetting. Dilute it (12 µL with 2 mL of Reagent D).

Standard:

Prepare in 1.5 mL tubes, the following SOD standard Solutions with Phosphate Buffer pH 7.4 as diluent (not included):1U/mL; 0.1U/mL; 0.05U/mL; 0.01U/mL and 0.001U/mL.



Scheme 2. *Standard curve preparation*

Assay Protocol

Considering a 96 well plate:

1. Pipette 20 µL of sample to each sample and Blank C well (you must prepare one Blank C well for each sample or standard).
2. Pipette 20 µL of ddH2O to Blank A and Blank B wells.
3. Add 200 µL of the Working Solution (previously prepared) to each well.
4. Add 20 µL of Reagent D to Blank B and to each Blank C.
5. Add 20 µL of the Enzyme Solution (previously prepared) to Blank A well and to each sample well (Is important to add Enzyme Solution at the same time in all wells, using for example a multichannel pipette). Then mix the plate thoroughly.
6. Incubate the plate at 37ºC for 20 min.
7. Read the absorbance at 450 nm using a microplate reader.

	Blank A	Blank B	Sample/Standard	Blank C
Sample/Standard			20 µL	20 µL
Working Solution	200 µL	200 µL	200 µL	200 µL
Enzyme Solution	20 µL			
Reagent D		20 µL		20 µL
ddH_2O	20 µL	20 µL		

Sheme 3. *Instructions to fill the 96 well plate*

Data Analysis

1. Plot the % of inhibition at 450 nm of standards as function of their final concentrations (Scheme 2).

SOD Activity (% inhibition) = {[(ABlank A – ABlank B) - (Asample/standard – ABlankC)] / (ABlankA– ABlankB)} x100

2. Create a standard curve by plotting the % inhibition at 450 nm for each standard against the concentration of SOD standards.

3. Calculate the SOD activity of the samples using the equation obtained from the linear regression of the standard curve replacing the % inhibition values for each sample.

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Said refund or replacement is conditioned on buyer giving written notice to Bioquochem within 30 days after arrival of the material at its destination.

Expiration date: 1 year from the date of delivery

For further details, please refer to our website www.bqckit.com.