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A Geno Technology, Inc. (USA) brand name

# SOD Activity Assay

(Cat. # BAQ077, BAQ078, BAQ079)



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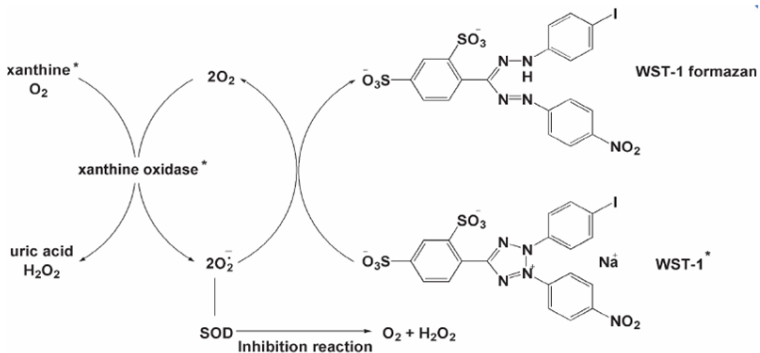
## INTRODUCTION

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.

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



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.

## ITEM(S) SUPPLIED

Description	Cat. # BAQ077 100 tests (96 well plate)	Cat. # BAQ078 200 tests (96 well plate)	Cat. # BAQ079 400 tests (96 well plate)
SOD Reagent A	2 mL	4 mL	8 mL
SOD Reagent B	20 µL	40 µL	80 µL
SOD Reagent C	40 mL	80 mL	160 mL
SOD Reagent D	10 mL	20 mL	40 mL
SOD Standard (3U/mL)	50 µL	100 µL	200 µL

## STORAGE CONDITIONS

This kit is shipped on blue ice. Store the components at 4°C upon arrival. If stored and used as directed this kit is stable for 12 months.

## ADDITIONAL ITEMS REQUIRED

- Spectrophotometer microplate reader that can measure 450 nm
- 96 well microtiter plate for microplate assay.
- 1.5ml Tubes
- Phosphate Buffer pH 7.4

## PREPARATION BEFORE USE

### For 100 assays

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

### Working Reagent

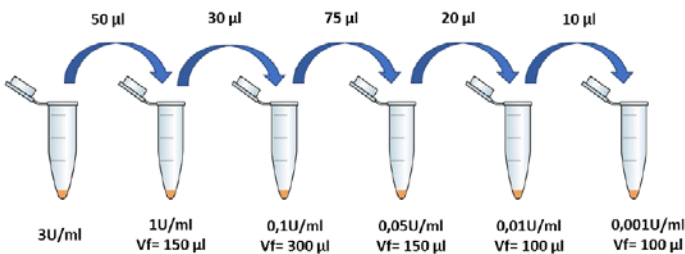
Mix 2 mL of SOD Reagent A with 38 mL of SOD Reagent C.

### Enzyme Solution

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



## PROTOCOL

1. For each sample to be assayed a “Sample Blank” must be used. Pipette 20 µL of sample to each sample and “Sample Blank” well.
2. Pipette 20 µL of ddH<sub>2</sub>O to Control and “Control Blank” wells.
3. Add 200 µL of the Working Solution to each well.
4. Add 20 µL of Reagent D to “Control Blank” and to each “Sample Blank”.
5. Add 20 µL of the Enzyme Solution to Control well and to each sample well. It is recommended to add at the same time to all wells, so a multi-channel pipette is recommended.
6. Mix the plate thoroughly.
7. Incubate the plate at 37°C for 20 min.
8. Read the absorbance at 450 nm using a microplate reader.

	Control	Control Blank	Sample/Standard	Sample Blank
Sample/Standard			20 µL	20 µL
Working Solution	200 µL	200 µL	200 µL	200 µL
Enzyme Solution	20 µL			
SOD Reagent D		20 µL		20 µL
ddH <sub>2</sub> O	20 µL	20 µL		

## DATA ANALYSIS

Plot the % of inhibition at 450 nm of standards as function of their final concentrations.

$$\% \text{ Inhibition} = \frac{(\text{Control} - \text{Ctrl Blank}) - (\text{Sample} - \text{Sample Blank})}{(\text{Control} - \text{Ctrl Blank})} \times 100$$

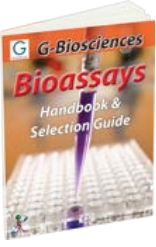
Where Sample is the sample to be assayed or the standard and Ctrl Blank is the Control Blank

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

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.

## RELATED PRODUCTS

Download our Bioassays Handbook.



<http://info2.gbiosciences.com/complete-bioassay-handbook>

For other related products, visit our website at [www.GBiosciences.com](http://www.GBiosciences.com) or contact us.



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