Product Information

Superoxide Dismutase (SOD) Activity Assay Kit

CS0009
Storage Temperature -20 °C

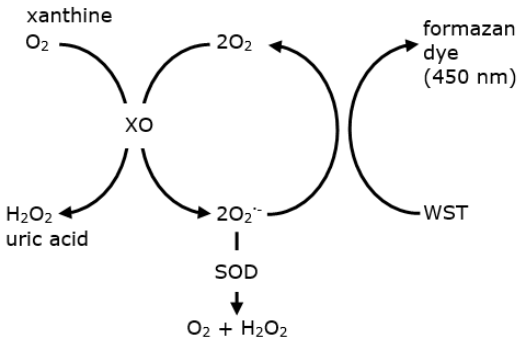
Product Description

Superoxide dismutases (SODs) are a family of metal ion-containing enzymes that are found in most organisms, from bacteria to mammals.[1] SODs catalyze the dismutation of superoxide (generated by aerobic respiration) to molecular oxygen and peroxide, by the following reaction:

$$2\ O^{\bullet-} + 2\ H^{+} \rightarrow H_2O_2 + O_2$$

SODs form the front line of defense against reactive oxygen species (ROS)-mediated injury, [2,3] and harbor anti-inflammatory activities.[4]

The Superoxide Dismutase (SOD) Activity Assay Kit provides a simple and sensitive procedure for measuring SOD enzymatic activity in various sample types. SOD activity is determined by measuring the decrease in superoxide anions (generated by the enzyme xanthine oxidase [XO]). Since superoxide anions interact with the provided WST dye, yielding color at 450 nm, the decrease in the color signal is proportional to SOD inhibition activity. The reaction is summarized below:



The kit allows the determination of SOD activity in percent inhibition, or in units of activity using the provided SOD Enzyme as a standard. The provided SOD Enzyme can also be used to screen for SOD inhibitors.

The kit's lowest limit of detection is 0.3 SOD units/mL.

This kit can be used to measure SOD activity in biological samples such as serum, plasma and erythrocytes, tissue homogenates and cell lysates.

Components

This kit contains sufficient reagents for 500 colorimetric tests.

Component	Component Number	Amount	Cap Color/ Container Information
Assay Buffer	CS0009A	100 mL	White cap/bottle
Dilution Buffer	CS0009B	50 mL	White cap/vial
SOD Enzyme	CS0009C	250 µL	Yellow cap/vial
WST	CS0009D	1 mL	Brown vial
Xanthine Oxidase	CS0009E	100 µL	Green cap/vial

Components Information

- Assay Buffer (CS0009A): Ready-to-use.
- Dilution Buffer (CS0009B): Ready-to-use.
- SOD Enzyme (CS0009C): 300 units/mL SOD enzyme solution from bovine liver. This component may be used as a positive control or to screen for SOD inhibitors. It may also be used as a standard to determine SOD activity in samples (see "standard curve preparation" below).
- WST (CS0009D): A 80X solution. Protect from light.
- Xanthine Oxidase [XO] (CS0009E): A 100X solution.

Equipment Required

(Not Provided)

- 96-well flat-bottom plates
- Plate reader equipped with a 450 nm (440-460 nm) filter.

Precautions and Disclaimer

For research 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

Upon receipt, store all components at -20 °C. WST (CS0009D) should be protected from light. The unopened kit is stable for 2 years as supplied.

Procedure

- All standards should be run in triplicate. Samples can be run either in duplicate or in triplicate.
- The assay is formatted for a 96-well microplate.
- Fresh working solutions should be prepared for every use. Unused working solutions should be discarded.
- Equilibrate all reagents to room temperature before use.
- Briefly centrifuge vials before opening.
- SOD activity can be measured either as percent inhibition activity, which does not require a standard curve, or by a direct comparison to the provided SOD Enzyme, which is used to prepare a standard curve (see "SOD standard curve preparation"). In case a standard curve is performed, a fresh set of standards should be prepared for every use.
- For convenience, an Excel-based calculation sheet is available on the Product Detail Page. Use this sheet to calculate the amounts of reagents required, as well as to calculate the test results.

Sample Preparation

All assays (samples, standards, controls and blank) require 20 µL of sample for each reaction (well). Therefore, bring the sample volume to 20 µL. When required, samples should be diluted in Dilution Buffer. For unknown samples, when using a SOD standard curve (see "SOD standard curve preparation" below), it is suggested to test several sample dilutions to ensure that the readings are within the linear range of the standard curve.

Blood samples

Plasma from citrated or EDTA-collected blood should be separated from erythrocytes by centrifugation at 4 °C (1,000 × *g* for 10 minutes). The plasma layer should be transferred to a new tube without disturbing the buffy layer. Store at -80 °C until the assay is performed. The recommended plasma dilution for the assay is 3-fold to 10-fold.

The remaining erythrocytes should be resuspended in 5 parts of ice-cold distilled water and centrifuged at 4 °C (10,000 × *g* for 10 minutes) to pellet the erythrocyte membranes. Transfer the supernatant to a new tube, and store at -80 °C until the assay is performed. Recommended erythrocyte lysate dilution for the assay is 100-fold.

Tissue

Tissue should be perfused with PBS to remove any erythrocytes. The tissue should be homogenized (For example, using a Dounce homogenizer) in ice-cold 0.1 M Trizma®-HCl, pH 7.4, containing 0.5 % Triton™ X-100, 5 mM mercaptoethanol, and protease inhibitors. Centrifuge the homogenate at 4 °C (14,000 × *g* for 5 minutes) and transfer the SOD-containing supernatant to a new tube.

Cells

Cells should be lysed in ice-cold 0.1 M Trizma®-HCl, pH 7.4, containing 0.5 % Triton™ X-100, 5 mM mercaptoethanol, and protease inhibitors. Centrifuge the lysate at 4 °C (14,000 × *g* for 5 minutes) and transfer the supernatant to a new tube.

Note: The supernatant contains total SOD activity from both cytosol and mitochondria. To separate the mitochondrial and cytosolic SOD activities, it is recommended to use Mitochondria/Cytosol Fractionation Kit (Cat. No. MIT1000).

SOD standard curve preparation:

The provided SOD Enzyme can be used to prepare a standard curve. The standard curve allows the measurement of SOD in units/mL. The linear range of the standard curve is 0.3-6 units/mL.

Unit Definition of the provided SOD Enzyme:
One unit will inhibit the rate of reduction of cytochrome c by 50% in a coupled system, using xanthine and xanthine oxidase, at pH 7.8 at 25 °C in a 3.0 mL reaction volume.

Prepare SOD working solution:
Dilute the SOD Enzyme 50-fold in Dilution Buffer to a final concentration of 6 units/mL, according to Table 1.

Table 1.

SOD working solution preparation

SOD Enzyme, CS0009C (yellow cap)	Dilution Buffer, CS0009B	Final Volume	Final SOC conc.
3 µL	147 µL	150 µL	6 units/mL

Add 0, 1, 2, 3, 5, 10 and 20 µL of the SOD working solution into a 96-well plate, generating 0 (No SOD - maximal absorbance), 0.3, 0.6, 0.9, 1.5, 3 and 6 units/mL standards. Bring the volume to 20 µL with Dilution Buffer, according to Table 2.

Table 2.

Standard curve preparation, per well*

SOD working solution volume (µL)	Dilution Buffer volume (µL)	SOD concentration	
		In standard (units/mL)	In well – final concentration (units/mL)
0	20	0.0	0.00
1	19	0.3	0.03
2	18	0.6	0.06
3	17	0.9	0.09
5	15	1.5	0.15
10	10	3.0	0.30
20	0	6.0	0.60

* Work in triplicate

WST working solution:

Prepare WST working solution according to Table 3.

160 µL of WST working solution is required for each reaction (well). Modify the volumes in Table 3 according to the number of wells in the assay.

Table 3.

WST working solution preparation, per 1 well

WST, CS0009D (brown vial; 80X)	Assay Buffer, CS0009A	Final volume
2 µL	158 µL	160 µL

Xanthine oxidase working solution:

Prepare xanthine oxidase working solution according to Table 4. 20 µL of xanthine oxidase working solution is required for each reaction (well). Modify the volumes in Table 4 according to the number of wells in the assay.

Table 4.

Xanthine oxidase working solution preparation, per 1 well.

Xanthine Oxidase, CS0009E (green cap; 100x)	Dilution Buffer, CS0009B	Final volume
0.2 µL	19.8 µL	20 µL

Assay reaction:

The following controls should be included in each assay:

- No SOD: No SOD sample or standard – maximal absorbance. **Note:** This control is included in the SOD standard curve preparation.
- No XO: Background color signal. This control may be included only when the sample solution has visible color that might interfere with the color signal of the assay.
- Blank: No sample and no xanthine oxidase – minimal background absorbance.

1. Add 20 µL of sample to each sample well. If a SOD standard curve was prepared, prepare a standard curve as described above (see: "Standard curve preparation").
2. Add 20 µL Dilution Buffer to No SOD and No XO (if necessary) wells, and 40 µL Dilution Buffer to Blank wells.
3. Add 160 µL of WST working solution to each sample, standard, control and blank well.
4. Initiate the reaction by adding 20 µL of xanthine oxidase working solution to each sample and standard well, and to the No SOD control wells. **Note:** Do not add xanthine oxidase to No XO or Blank wells.
5. Incubate the plate at 20-25 °C for 30 minutes.
6. Read the absorbance at 450 nm using a microplate reader.

A summary of the assay reaction setup is outlined in Table 5.

Table 5.

Reaction volumes for sample, standard and blanks

		Controls		
	Sample/ Standard	No SOD	No XO	Blank
SOD sample or standard	20 µL	--	20 µL	--
WST working solution	160 µL	160 µL	160 µL	160 µL
Xanthine oxidase working solution	20 µL	20 µL	--	--
Dilution Buffer	--	20 µL	20 µL	40 µL

Results

Calculations:

An Excel-based calculation sheet is available at the Product Detail Page. Use this sheet to calculate the test results.

If the Excel-based calculation sheet at the Product Detail Page is not used, calculations should be performed as follows:

To calculate the SOD inhibition rate percentage

$$\text{SOD activity*} = \frac{(A - B) - (C - D^{**})}{(A - B)} \times 10$$

* Inhibition rate percentage

Where:
A: Absorbance value of No SOD control

B: Absorbance value of Blank

C: Absorbance value of sample

D: Absorbance value of No XO.

(** Note: If No XO control is not required, use "B" (Blank) instead.)

To calculate SOD activity in units/mL

1. Calculate the average absorbance of each standard, sample and control, and subtract the Blank value from all standards, samples and controls values.
7. To obtain the linearized SOD rate, divide No SOD absorbance (maximal absorbance) by itself and by all other blank-subtracted standards and samples absorbance readings. This is the linearized SOD rate.
8. Plot the SOD standard concentrations (in units/mL) against the linearized SOD rate of all standards.

Calculate the activity of the SOD in the sample

$$\text{SOD (units/mL)} = \frac{(\text{Sample}_{LSR} - \text{intercept})}{\text{Slope}} \times 10 \times DF$$

Where:
Sample_{LSR} = linearized SOD rate of the sample

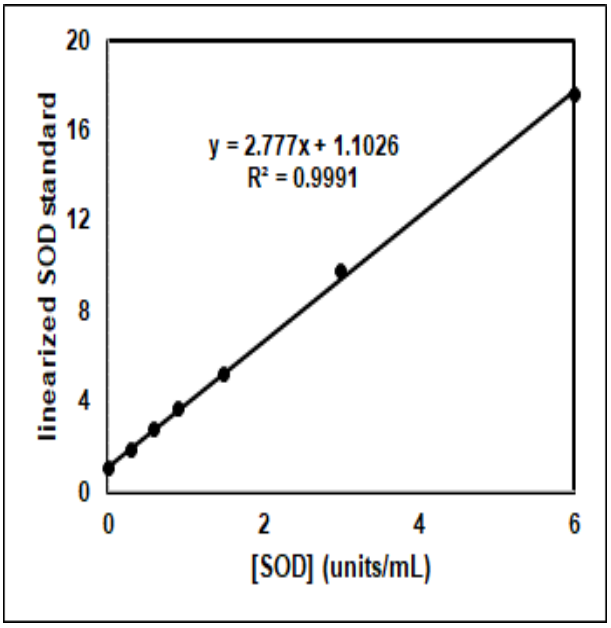
DF = Sample dilution factor.

Note: multiply by 10 to account for the reaction dilution within the well (20 mL sample in a final reaction volume of 200 mL)

See Figure 1 for a typical SOD standard curve.

Figure 1.

SOD standard curve. SOD Enzyme (CS0009C) was used to construct a standard curve of the linearized SOD rate.



References

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