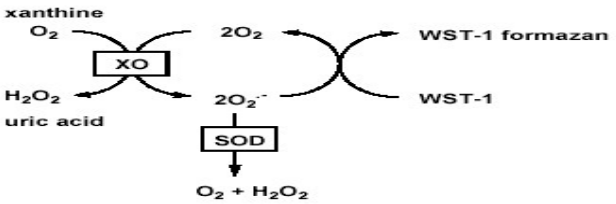
Superoxide Dismutase (SOD) Activity Assay Kit

(Catalog #BN00601; 100 assays; Store kit at 0-5° C)

I. Introduction:

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.



Kit Contents:

Component	BN00601 100 assays	Cap Color	Part Number
WST Solution	1 ml	Red	BN00601-1
SOD Enzyme Solution	20 µl	Green	BN00601-2
SOD Assay Buffer	20 ml	WM	BN00601-3
SOD Dilution Buffer	10 ml	NM	BN00601-4

II. Reagent Preparation and Storage Conditions:

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°C.

Enzyme Working Solution: Centrifuge the 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 with 2.5 ml of Dilution Buffer. The diluted enzyme solution is stable for up to 3 weeks at 4°C.

III. Sample Preparation:

1. **Blood samples:** Collect blood using citrate or EDTA. Centrifuge at 1,000 x g for 10 min at 4°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 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°C until ready for analysis. Plasma can be diluted approx. 3 - 10x and the red cell lysate diluted approx. 100X prior to SOD assay.
2. **Tissue and cells:** Tissue should be perfused with PBS or 150 mM KCl 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 β-ME, 0.1 mg/ml PMSF. Centrifuge the crude tissue homogenate/cell lysate at 14000 x g for 5 minutes at 4°C and discard the cell debris. The supernatant contains total SOD activity from cytosolic and mitochondria.
   If it is desired to measure SOD activity from cytosol and mitochondria separately, cytosol and Mitochondria can be separated by using Assay Genie's BN00601 Mitochondrial/Cytosol Fractionation Kit. SOD activity is then measured from the Mitochondria and Cytosol fractions separately.

IV. SOD Assay Protocol:

*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.

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 I).
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°C for 20 minutes.
6. Read the absorbance at 450 nm using a microplate reader.
7. Calculate the SOD activity (inhibition rate %) using the following equation.

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

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

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

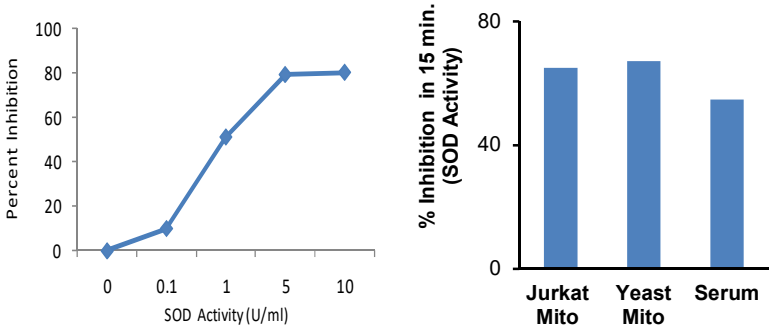


Figure: SOD Activity (% inhibition rate): human serum (10 µl) and isolated mitochondria from Jurkat cells (10 µg), and yeast (*Saccromyces cerevisiae, 100 ug*), was used to determine SOD Activity according to the kit protocol. Activity was measured in 15 min. at 37°C.

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		