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Product Information

Superoxide Dismutase (SOD) Activity Assay Kit

I. Kit Contents:

Components	K2035-100 100 assays	Cap Color	Part Number
WST Solution	1 ml	Red	K2035-C-1
SOD Enzyme Solution	20 µl	Green	K2035-C-2
SOD Assay Buffer	20 ml	WM	K2035-C-3
SOD Dilution Buffer	10 ml	NM	K2035-C-4

II. Introduction:

Superoxide dismutase (SOD) is an important antioxidative enzyme that catalyzes the dismutation of the superoxide anion into hydrogen peroxide (H_2O_2) or molecular oxygen (O_2).

The Superoxide Dismutase (SOD) Activity Assay Kit provides a sensitive and convenient way for detection of Superoxide Dismutase (SOD) activity based on a colorimetric method in a variety of biological fluids. When reduction with superoxide anion, WST-1 produces a water-soluble formazan dye, which can be easily detected at 450 nm using a spectrophotometer or an ELISA plate reader. The rate of the reduction with a superoxide anion is linearly correlated to the xanthine oxidase (XO) activity, and is inhibited by SOD.

III. 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℃.

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℃.

IV. Sample Preparation:

1. Blood samples: Collect blood using citrate or EDTA. Centrifuge at 1,000 x g for 10 min at 4℃. Transfer the plasma layer to a new tube without disturbing the buffy layer and store at -80℃ 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℃ 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℃ 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 BioVision K256-100 Mitochondrial/Cytosol Fractionation Kit. SOD activity is then measured from the Mitochondria and Cytosol fractions separately.

V. SOD Assay Protocol:

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℃ 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.

SOD Activity (inhibition rate %) = [(Ablank1 – Ablank3) – (Asample – Ablank2)] /(Ablank1 – Ablank3) x 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

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the Nucleotide releasing buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures

		• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • 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.		

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