Superoxide Dismutase (SOD) Activity Assay Kit

1 About This Kit

Superoxide dismutases (SODs), as a group of the most important antioxidative enzymes, catalyze the dismutation of the superoxide anion free radical ($^{\cdot}O_2^-$) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2). SODs are metalloenzymes and exist in three forms in humans: cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD (Cu/Zn).

This product evaluates the activity of all SODs by employing hypoxanthine and xanthine oxidase to generate superoxide radicals which react with the WST-1 tetrazolium salt and form a yellow formazan dye. The formation rate of the formazan is inhibited by the presence of SODs. One unit of SOD causes 50% inhibition in the rate of the reduction of WST-1 under the assay condition, therefore, the activity of SOD or SOD-like materials is then measured by the degree of inhibition of this reaction.

$$\text{Hypoxanthine} \xrightarrow{\text{Xanthine Oxidase}} \text{Uric Acid} + O_2\cdot$$

$$\text{WST-1} \xrightarrow{O_2\cdot} \text{Formazan dye} \qquad \underline{\text{OR}} \qquad O_2\cdot^- + O_2\cdot^- + 2H^+ \xrightarrow{\text{SOD}} O_2 + H_2O_2$$

Kit Components

Item Label	Amount	
	48 Tests Kit	96 Tests Kit
WST-1 Solution	1 ml	1 ml (2 vials)
Enzyme Solution	15 µl	15 µl (2 vials)
Buffer Solution	20 ml	20 ml (2 vials)
Dilution Buffer	12 ml	12 ml
96 Well Microplate	1 Plate	2 Plates
Instruction Manual	1 Manual	1 Manual

Storage and Stability

- This kit will perform as specified if **stored at 4°C**.
- Use before the **expiration date** indicated on the box.

Kit Performance

- **Precision:** Human SOD with 8 replicates on 3 different days showed the intra and inter assay coefficient of variation 6.0% and 7.1% respectively.
- **Sensitivity:** 0.2 U/mL
- **Detection range:** 0.2 – 14.4 U/mL
- **Application:** For *in vitro* measurement of the activity of all SOD enzyme isoforms (Cu/Zn, Mn and Fe SOD) in serum, plasma, tissue, cell lysates and other biological fluids.

Materials Required (Not Provided)

- Double distilled water (ddH2O)
- Microtubes, 15 mL/ 50 mL tubes.
- Dounce homogenizer (if using tissue samples)
- PBS 0.01 M, pH 7.4 (for sample dilution preparation and tissue homogenization)

Required Instrumentation

- Multiwell microplate reader (capable of measuring absorbance between 440-460 nm)
- Microfuge

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- Incubator (37°C)
- Pipettes with variable volume setting

Warning and Precautions

- It is recommended that gloves, lab coat, and protective eyewear be worn at all times.
- In case of contact with skin or eyes, wash thoroughly with soap and cold water.
- Consider all components as hazardous and dispose according to established safety laws.

Sample Preparation

Note: Assay all samples immediately upon extraction or store at -80°C for up to 1-2 months.
Note: Thaw samples on ice (Regulation of temperature at 4°C increases sensitivity and reproducibility).

PLASMA AND BLOOD SAMPLES

1. Collect whole blood using an anticoagulant such as heparin, EDTA, or citrate.
2. Centrifuge at 1,000 x g for 10 minutes at 4°C.
3. Carefully pipette off the ***plasma*** layer without disturbing the buffy layer, transfer to fresh tubes and place on ice.
4. Remove the buffy layer from the red cell pellet.
5. Resuspend the erythrocytes in four times its volume of ice-cold distilled water.
6. Centrifuge at 10,000 x g for 15 minutes at 4°C.
7. Collect the supernatant (***erythrocyte lysate***) and transfer to appropriate size tubes and place on ice.

SERUM

1. Collect whole blood without using an anticoagulant.
2. Allow blood to clot for 30 minutes at 25°C.
3. Centrifuge the blood at 2000 x g for 15 minutes at 4°C and
4. Carefully pipette off the yellow serum layer without disturbing the buffy layer and transfer to new tubes and place on ice.

TISSUE HOMOGENATES

1. Weight out the amount of tissue necessary for each assay (10-100 mg wet tissue).
2. Perfuse/rinse the tissue with PBS or 150 mM KCl, to remove any red blood cells and clots.
3. Homogenize the tissue in 1 ml ice cold PBS or 150 mM KCl.
4. Centrifuge at 14,000 x g for 5 minutes at 4°C.
5. Collect the supernatant and transfer to fresh tubes and place on ice.

Note: RIPA buffer (without SDS) supplemented with PMSF protease inhibitors can also be used as lysis buffer.

Note: If it is desired to measure SOD activity from cytosol and mitochondria separately, tissue/cell samples can be prepared according to method described by *Mattiazzi et al* (2002). *J biological chem*. 277: 29626-33.

CELL LYSATES

1. Harvest the number of cells necessary for each assay (~2 x 10^6 cells/ml recommended). For harvesting adherent cells, don't use proteolytic enzymes (like trypsin); rather use a rubber policeman.
2. Lyse cells by repeated cycles of freezing and thawing or sonication in ice cold PBS (pH 7.2-7.4).
3. Centrifuge at 14,000 x g for 5 minutes at 4°C.

4. Collect the supernatant and transfer to fresh tubes and place on ice. For long-term storage, store in working aliquots at –80 °C.

RECOMMENDED DILUTION FACTOR FOR DIFFERENT SAMPLE TYPES

Table 1: (for reference only)

Note: It is advised to take 2 or 3 samples and perform a pre-assay by preparing a series of dilutions and determine the dilution factor when the SOD inhibition ratio is 25%~65% (the optimal inhibition ratio is the range of 40%~60%.) before your formal assay.

Note: Dilute samples with PBS (0.01M, pH 7.4)

Sample type	Dilution factor
Human serum / Plasma	3-5
Rat serum	20-30
Urine	1
Red Blood Cell lysate	100
10% Rat liver tissue homogenate	340-370
10% Rat heart tissue homogenate	80-100
10% Rat kidney tissue homogenate	100-120
10% Rat brain tissue homogenate	50-100
10% Plant tissue homogenate	5-10

Reagent Preparation

1) **Working Solution**: Dilute 1 mL of WST-1 Solution with 19 mL of Buffer Solution. *Working solution* is stable for 2 months if stored at 4°C in dark.
2) **Ready Enzyme Solution:** Centrifuge the Enzyme Solution vial for 5 seconds. Dilute one vial of Enzyme Solution with 1200 µL of Dilution Buffer to perform 48 tests or prepare both vials for 96 tests. *Ready Enzyme Solution* is stable up to 1 month at 4°C.

Note: The Enzyme Solution has 2 layers and if you intend to take lesser volumes from its vial, it must be mixed well before pipetting.

Things to Note

- Equilibrate all prepared reagents and samples to room temperature just prior to use, and gently agitate for homogenization.
- Assay all samples and controls in duplicate/triplicate.
- It is recommended not to assay a large number of samples in a single run.
- Use multiple channel pipettes to avoid reaction time lag of each well.
- SOD activities between different samples can be normalized on protein Conc. or cell No.
- If necessary, the provided Dilution Buffer can be used for diluting samples.
- The SOD inhibition ratio can reach to 100%. If so, dilute and re-assay.
- Bovine Serum Albumin: 5% w/v, Ascorbic acid: 0.1 mM and Glutathione, reduced form: 5 mM can cause 10% increase in the O.D. value and is considered as interfering reagents.

Assay Protocol

As described below, add samples and reagents to appointed well.

Component	sample solution (µL)	sample sol. blank (µL)	blank 1 (µL)	blank 2 (µL)
Sample solution	20	20	0	0
ddH2O	0	0	20	20
Working solution	200	200	200	200
Ready enzyme solution	20	0	20	0
Dilution Buffer	0	20	0	20

Note: The term "sample solution" refers to diluted samples that have to be prepared before the formal assay based on the condition and type of sample (see **Table 1** for reference).

1. Add 20 μL of each sample solution to assigned sample and sample blank wells.
2. Add 20 μL ddH2O to each blank 1 and blank 2 wells.
3. Add 200 μL of *Working Solution* into each well.
4. Add 20 μL of Dilution Buffer to all sample blank and blank 2 wells.
5. Add 20 μL of *Ready enzyme solution* to each sample well and Blank1 wells. Perform this step as quickly as possible (*multi-channel pipettes are preferred at this step*).
6. Shake for 15 seconds and incubate at 37°C for 20 minutes.
7. Read the absorbance at 440-460 nm using a plate reader.

Calculations

Definition: when SOD inhibition ratio in this reaction system reaches 50%, the corresponding enzyme level is 1 SOD activity unit (U).

FORMULA FOR SERUM/PLASMA SAMPLES

$$\text{Inhibition ratio of SOD (\%)} = \frac{(A\ blank1 - A\ blank2) - (A\ sample - A\ sample\ blank)}{(A\ blank1 - A\ blank2)} \times 100$$

A = Absorbance

$$\text{SOD Activity (U/ml)} = Inhibition\ ration\ of\ SOD\ (\%) \div 50\% \times \left(\frac{240\ \mu l}{20\ \mu l}\right) \times dilution\ factor\ of\ sample$$

FORMULA FOR TISSUE AND CELLS

$$\text{Inhibition ratio of SOD (\%)} = \frac{(A\ blank1 - A\ blank2) - (A\ sample - A\ sample\ blank)}{(A\ blank1 - A\ blank2)} \times 100$$

A = Absorbance

$$\text{SOD Activity (U/mg prot)} = Inhibition\ ration\ of\ SOD\ (\%) \div 50\% \times \left(\frac{240\ \mu l}{20\ \mu l}\right) \times dilution\ factor\ of\ sample \div protein\ concentration\ of\ sample\ (mg\ \ prot/ml)$$

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Absorbance <0.05 in sample wells	High concentration of SOD	Dilute samples with Dilution Buffer and re-assay
	WST-1 Solution was not added to Working solution	Re-assay with complete Working solution
No activity was detected in samples	Sample was too dilute	Re-assay with lower dilutions
Erratic values; dispersion of duplicate/triplicates	Poor pipetting/technique	Be careful not to splash the contents of the wells
	Bubble in wells	Remove bubbles by tapping