Superoxide Dismutase (SOD) Assay Kit

Catalog No.: abx096009

Size: 100 Assays

Storage: Store the Enzyme Stock Solution at -20°C and all other components at 4°C in the dark.

Application: For quantitative detection of Superoxide Dismutase activity in serum, plasma, tissue homogenates, cell culture supernatants, urine, and other biological fluids.

Detection Range: 0.2 U/ml – 14.4 U/ml

Sensitivity: 0.2 U/ml

Introduction: Superoxide Dismutases (SODs) are enzymes that catalyze the dismutation of superoxide radicals (O_2^-) to hydrogen peroxide (H_2O_2) and oxygen (O_2). Superoxide radicals can cause damage to cells, and therefore SODs plays an important role as an antioxidant. In mice, deficiencies in SOD1 and SOD2 have been linked to tumor formation and liver cancer, whilst over-expression of SODs protects tumor cells from apoptosis.

Abbexa's Superoxide Dismutase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating total superoxide dismutase activity. Xanthine Oxidase (XOD) catalyzes the formation of uric acid and hydrogen peroxide from xanthine, and also the formation of NBT-diformazan from NBT and superoxide. SODs reduce superoxide concentration and therefore lowers the rate of NBT-diformazan formation. NBT-diformazan concentrations can be determined by measuring the absorbance at 450 nm, from which superoxide dismutase activity can be calculated.

Kit components

1. 96 well microplate
2. Assay Buffer: 24 ml
3. Substrate: 0.14 ml
4. Enzyme Stock Solution: 0.3 ml
5. Enzyme Diluent: 2 × 1.5 ml
6. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (450 nm) and incubator
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Double distilled water
5. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
6. Timer
7. Ice
8. Sonicator
9. Mortar

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Substrate Solution**
  Dilute Substrate with Assay Buffer 201-fold to prepare the Substrate Solution (for example, add 0.1 ml of Substrate to 20 ml of Assay Buffer and mix thoroughly). The resulting solution may be stored at 4°C for up to 7 days.

- **Enzyme Solution**
  Dilute Enzyme Stock Solution with Enzyme Diluent 11-fold to prepare the Substrate Solution (for example, add 0.1 ml of Enzyme to 1 ml of Enzyme Diluent and mix thoroughly). The resulting solution may be stored at 4°C for up to 3 days.

  Note: *Enzyme Stock Solution should be thawed slowly on ice. Avoid repeated freeze-thaw cycles.*

2. Sample

- **Cell culture supernatants**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge at 1000 × g to precipitate the cells. Discard the supernatant and add 1 ml of Homogenization Medium (Normal saline, 0.9% NaCl; or PBS 0.01 M, pH 7.4) for every 5,000,000 cells. Sonicate on ice at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 1500 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

- **Tissue samples**
  Weigh 0.1 g of sample, and wash thoroughly with PBS to remove excess blood. Homogenize sample in 1 ml of Homogenization Medium (Normal saline, 0.9% NaCl; or PBS 0.01 M, pH 7.4) on ice. Centrifuge at 10,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately. The protein concentration of the supernatant should be determined separately.

- **Serum samples**
  Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 1000 × g for 20 mins. If precipitate appears, centrifuge again. Assay immediately or aliquot and store at -20°C or -80°C.

- **Plasma samples**
  Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 mins at 1000 x g, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples.

- **Urine and other biological fluids**
  Collect fresh sample, and centrifuge at 10,000 × g for 15 minutes at 4°C. Take the supernatant on ice and assay immediately or aliquot and store at -20°C or -80°C.

  Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

  We recommend carrying out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with distilled water, then carry out the assay procedure and calculate the inhibition ratio of the samples. The optimal dilution factor is an inhibition ratio in the range of 25% - 65%, where the inhibition ratio can be calculated as:

$$\textbf{Inhibition Ratio}\ (\%) = \frac{(\mathbf{OD_{Control}} - \mathbf{OD_{Blank}}) - (\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}})}{(\mathbf{OD_{Control}} - \mathbf{OD_{Blank}})} \times \mathbf{100}$$

  If the inhibition ratio is > 65%, the sample should be diluted further. If the inhibition ratio is < 10%, the sample concentration should be increased.

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

The following sample dilutions are intended as a guide, the optimal dilution factor should be determined by the end user. Dilute using Normal saline, 0.9% NaCl; or PBS 0.01 M, pH 7.4.

Sample	Dilution factor
Human Serum	3-5
Rat Serum	20-30
Urine	1
Human Hydrothorax	2
Cell culture supernatants	2-3
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
HepG2 cell homogenate (3 mg protein/ml)	30-40

B. Assay Procedure

Bring all reagents to room temperature prior to use.
If the expected concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured concentrations within the detection range of the kit.

1. Set sample, control and blank wells on the microplate and record their positions. We recommend setting up each sample and control in duplicate.
2. Add 20 µl of Double distilled water to the control and blank wells.
3. Add 20 µl of sample to each sample well.
4. Add 20 µl of Enzyme Solution to the control and sample wells.
5. Add 20 µl of Enzyme diluent to the blank wells
6. Add 200 µl of Substrate Solution to each well.
7. Tap the plate gently to mix. Incubate at 37°C for 20 minutes.
8. Read and record the absorbance at 450 nm.

C. Calculations

One Unit (U) of SOD activity is defined as the activity of SOD when the inhibition ratio, i, is 50%.

$$\textbf{Inhibition Ratio (i \%)} = \frac{(\mathbf{OD_{Control}} - \mathbf{OD_{Blank}}) - (\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}})}{(\mathbf{OD_{Control}} - \mathbf{OD_{Blank}})} \times \mathbf{100}$$

1. Superoxide Dismutase activity per mass of protein (in mg):

$$\textbf{SOD (U/mg protein)} = \frac{f}{\mathbf{C_{Protein}}} \times \frac{\mathbf{V_{Total}}}{\mathbf{V_{Sample}}} \times \frac{\mathbf{i}}{\mathbf{50}} = \frac{f}{\mathbf{C_{Protein}}} \times \mathbf{0.24} \times \mathbf{i}$$

2. Superoxide Dismutase activity per volume of sample (in ml).

$$\textbf{SOD (U/ml)} = f \times \frac{\mathbf{V_{Total}}}{\mathbf{V_{Sample}}} \times \frac{\mathbf{i}}{\mathbf{50}} = f \times \mathbf{0.24} \times \mathbf{i}$$

where:

$\mathbf{OD_{Control}}$	Absorbance of control well
$\mathbf{OD_{Blank}}$	Absorbance of blank well
$\mathbf{OD_{Sample}}$	Absorbance of sample well
$\mathbf{C_{Protein}}$	Concentration of protein (mg/ml)
$\mathbf{i}$	$\textbf{Inhibition Ratio (i \%)} = \frac{(\mathbf{OD_{Control}}-\mathbf{OD_{Blank}})-(\mathbf{OD_{Sample}}-\mathbf{OD_{Blank}})}{(\mathbf{OD_{Control}}-\mathbf{OD_{Blank}})} \times \mathbf{100}$
$\mathbf{V_{Total}}$	Total volume of reaction (240 µl)
$\mathbf{V_{Sample}}$	Volume of sample (20 µl)
f	Sample dilution factor prior to assay