Superoxide Dismutase Assay Kit

Catalog Number KA3782
100 assays
Version: 02

Intended for research use only
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Introduction

Intended Use

- Application
  ✓ Determination of SOD in blood, cell, tissue and other biological samples.

- Features:
  ✓ Sensitive and accurate. Linear detection range of 0.05 - 3 U/mL SOD.
  ✓ Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. No wash and reagent transfer steps are involved. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

Background

SUPEROXIDE DISMUTASES (SOD, EC1.15.1.1) are enzymes that catalyze the dismutation of superoxide into O\textsubscript{2} and H\textsubscript{2}O\textsubscript{2}. They are an important antioxidant defense in all cells exposed to O\textsubscript{2}. There are three major families of superoxide dismutase: Cu/Zn, Fe/Mn, and the Ni type. Aberrant SOD activities have been linked to diseases such as amyotrophic lateral sclerosis, perinatal lethality, neural disorders and cancer.

Superoxide Dismutase Assay Kit provides a convenient colorimetric means for the quantitative determination of SOD enzyme activity in biological samples. In the assay, superoxide (O\textsubscript{2}\textsuperscript{−}) is provided by xanthine oxidase (XO) catalyzed reaction. O\textsubscript{2}\textsuperscript{−} reacts with a WST-1 dye to form a colored product. SOD scavenges the O\textsubscript{2}\textsuperscript{−} thus less O\textsubscript{2}\textsuperscript{−} is available for the chromogenic reaction. The color intensity (OD\textsubscript{440}nm) is used to determine the SOD activity in a sample.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer</td>
<td>20 mL</td>
</tr>
<tr>
<td>SOD Enzyme</td>
<td>120 µL</td>
</tr>
<tr>
<td>Xanthine</td>
<td>600 µL</td>
</tr>
<tr>
<td>Diluent</td>
<td>20 mL</td>
</tr>
<tr>
<td>XO Enzyme</td>
<td>120 µL</td>
</tr>
<tr>
<td>WST-1</td>
<td>600 µL</td>
</tr>
</tbody>
</table>

Storage Instruction

Store all reagents at -20°C. Shelf life of 6 months after receipt.

Materials Required but Not Supplied

Pipetting devices, tissue homogenizer, centrifuge and tubes, clear flat-bottom 96-well plates and plate reader.

Precautions for Use

Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents.
Assay Protocol

Sample Preparation

Note: If not assayed immediately, samples can be stored at -80°C for one month. All samples can be diluted in 50 mM potassium phosphate, pH 7.4.

- Tissue samples. Perfuse tissue with cold PBS to remove any red blood cells. Homogenize tissue at 5 mL/g in cold lysis buffer (50 mM potassium phosphate, 0.1 mM EDTA, 0.5% Triton X-100). Centrifuge at 12,000 g for 5 minutes at 4°C. Use supernatant for total SOD assay.

- Cell samples.
  ✓ Suspension cells: Centrifuge 1-2 x 10^6 cells at 800 g for 2 minutes and discard supernatant. Wash cells with cold PBS, centrifuge, and discard the supernatant. Resuspend cells in 0.5 mL of cold lysis buffer. After 10 min on ice, centrifuge at 12,000 g for 5 min. Use supernatant for total SOD assay.
  ✓ Adherent cells: Wash 1-2 x 10^6 cells cold PBS. Place dish on ice. Add 0.5 mL of cold lysis buffer. After 10 min on ice, collect cells/debris with a rubber policeman. Centrifuge the cell extract at 12,000 g for 5 min. Use supernatant for total SOD assay.
  Note: if it is desired to determine cytosolic and mitochondrial SOD activities separately, tissue/cell samples can be prepared according to Mattiazzi et al (2002). JBC 277: 29626-33.

- Blood samples: Collect serum, or plasma (heparin, citrate or EDTA) using standard protocols. The erythrocyte pellet can be lysed in 5x volume of cold dH_2O; centrifuge at 12,000 g for 5 min to pellet the erythrocyte membranes. Dilute serum/plasma 1:5, red cell lysate 1:100 prior to SOD assay.

Prior to assay, bring all reagents to room temperature (25°C). The Xanthine reagent may appear to be turbid. Briefly vortex this tube before pipetting. Briefly centrifuge enzyme tubes, keep on ice during assay.
**Assay Procedure**

1. Standards. Mix 8µL SOD Enzyme with 392µL Diluent to give 3U/mL SOD standard. Dilute standards as below.

<table>
<thead>
<tr>
<th>No</th>
<th>3U/mL SOD + Diluent</th>
<th>Standard (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100 µL + 0 µL</td>
<td>3.0</td>
</tr>
<tr>
<td>2</td>
<td>80 µL + 20 µL</td>
<td>2.4</td>
</tr>
<tr>
<td>3</td>
<td>60 µL + 40 µL</td>
<td>1.8</td>
</tr>
<tr>
<td>4</td>
<td>40 µL + 60 µL</td>
<td>1.2</td>
</tr>
<tr>
<td>5</td>
<td>18 µL + 82 µL</td>
<td>0.54</td>
</tr>
<tr>
<td>6</td>
<td>8 µL + 92 µL</td>
<td>0.24</td>
</tr>
<tr>
<td>7</td>
<td>4 µL + 96 µL</td>
<td>0.12</td>
</tr>
<tr>
<td>8</td>
<td>0 µL + 100 µL</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Transfer 20 µL SOD standards to separate wells of a clear flat-bottom 96-well plate. Transfer 20 µL samples to separate wells.

2. Prepare enough Working Reagent for the standard and sample wells.
   - For each well, mix 160 µL Assay Buffer, 5 µL Xanthine and 5 µL WST-1. Transfer 160 µL Working Reagent to each well and tap plate to mix.
   - For each well, dilute the XO Enzyme 1:20 in Diluent. Quickly add 20 µL diluted XO enzyme to each assay well (use of a multi-channel pipettor is recommended). Tap plate to mix.

3. Immediately read OD<sub>440nm</sub> (OD<sub>420-460nm</sub>) (OD<sub>o</sub>). Incubate for 60 min at room temperature (25°C) in the dark. Read OD<sub>440nm</sub> again (OD<sub>60</sub>).


Data Analysis

Calculation of Results

✓ For each standard and sample well, calculate $\Delta OD_{60} = OD_{60} - OD_0$.
✓ Calculate $\Delta OD = \Delta OD_{std8} - \Delta OD$ for each standard and sample where $\Delta OD_{std8}$ is the $\Delta OD$ for Standard # 8 (the standard with no SOD activity and highest possible absorbance).
✓ Plot the Standard Curve $\Delta OD$ vs [SOD](U/mL). Use the $\Delta OD$ for sample to determine SOD activity of sample from the standard curve.
Resources

References

