Superoxide Dismutase Colorimetric Assay Kit

Catalog No. KM0146

Detection and Quantification of Superoxide Dismutase Concentrations in Biological Samples.

Research Purposes Only. Not Intended for Diagnostic or Clinical Procedures.
INTRODUCTION

The enzyme Superoxide Dismutase (SOD) catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. SOD functions as an important antioxidant defense and helps to deactivate the superoxide, one of the main cellular reactive oxygen species. It is widespread in nature, and variations have been found in virtually all oxygen-metabolizing cells. Treatment with SOD decreases reactive oxygen species generation and lessens oxidative stress. This can help neutralize precancerous cell changes and diminish effects of free-radicals like aging and neurodegenerative diseases. In addition, there is anti-inflammatory activity as well as other pharmacological activities.

This assay can measure SOD with a highly sensitive colorimetric method. Xanthine is converted to superoxide radical ions (O₂⁻), uric acid, and hydrogen peroxide by xanthine oxidase (XO). The superoxide is coupled to an enzyme reaction to generate a blue-colored product with absorbance at 560 nm when read by an absorbance microplate reader. SOD competes with this reaction for the superoxide substrate, reducing the signal. Thus, low signal at 560 nm correlates with high levels of SOD activity.

FEATURES

Number of Assays: 200 Assays
Colorimetric Dynamic Range: 0.03 to 100 U/ml Superoxide Dismutase
Absorbance at 560 nm

STORAGE & HANDLING

Upon receipt, store all components in the original box at -20°C, protected from light. The kit should be stable for at least six months.

Note: Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin.
KIT CONTENTS & STOCK PREPARATION

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount</th>
<th>Storage</th>
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<tbody>
<tr>
<td>96-Well Microplate</td>
<td>2 plates</td>
<td>RT</td>
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<tr>
<td>Reaction Buffer</td>
<td>20 ml</td>
<td>-20°C</td>
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<tr>
<td>Superoxide Dismutase Standard</td>
<td>1 vial of 500 U</td>
<td>-20°C</td>
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<tr>
<td>Enzyme Mix</td>
<td>2 Vials</td>
<td>-20°C</td>
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<tr>
<td>Reaction Mix</td>
<td>1 Vial of 100 µl</td>
<td>-20°C</td>
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<tr>
<td>Dye Reagent</td>
<td>2 Vials</td>
<td>-20°C</td>
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Note: If not running 200 assays at once, aliquot and store reagents at -20°C. Use within 2 months.

MATERIALS NOT PROVIDED

Pipetting devices, tubes, and microplate reader.

ASSAY RESTRICTIONS

- This kit is intended for research purposes only, NOT diagnostic or clinical procedures of any kind.
- Materials included in this kit should NOT be used past the expiration date on the kit label.
- Reagents or substrates included in this kit should NOT be mixed or substituted with reagents or substrates from any other kits.
- Variations in pipetting technique, operator laboratory technique, kit age, incubation time or temperature may cause differences in performance of the materials provided.
- The assay is designed to eliminate interference and background by other cellular macromolecules or factors present within any biological samples. However, the possibility of background noise cannot be fully excluded until all factors have been tested using the assay kit.
REAGENT & SAMPLE PREPARATIONS FOR 1 PLATE
Please read the manual in its entirety before proceeding with the assay.

Note: Warm all reagents to room temperature before use and protect from light. Aliquot and store the reagents at -20°C if not all of it is used.

1. Preparation of Standard
Add 50 µl of Reaction Buffer to the Superoxide Dismutase Standard to obtain a 10 kU/ml stock concentration.

To prepare the standard, dilute the appropriate amount of Superoxide Dismutase stock solution in Reaction Buffer, starting from a high point of 100 U/ml down to a low point of 0.03 U/ml to create 7 concentrations for the standard. The last row consists of only Reaction Buffer to serve as a negative control. The standards are run in duplicate. Use a 96-well clear, flat bottom plate.

2. Preparation of Samples
Dilute the samples in Reaction Buffer. A variable dilution will be required depending on the total amount of Superoxide Dismutase present in your sample.

3. Preparation of Working Solution
Add 50 µl of Reaction Buffer to the Enzyme Mix and mix well. Then transfer 50 µl of this Enzyme Mix to 2.5 ml of Reaction Buffer to make the Working Solution. Mix well.

4. Preparation of Dye Reagent
Add 2.5 ml of Reaction Buffer into a Dye Reagent bottle and mix well. Then add 50 µl of Reaction Mix and mix well.

Note: The Dye Reagent should be prepared right before the experiment and kept away from light. This solution is not stable; the unused portion should be discarded.
ASSAY PROTOCOL FOR 1 PLATE

1. Add 50 µl of each sample/standard to each well in a 96-well flat bottom microplate. The standards are run in duplicate. It is advised to run the samples in duplicate.

Sample Plate Layout (you do not have to follow this)

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Note: BL=Blank Control, ST=Superoxide Dismutase Standards, EX=Experimental Test Samples

2. Add 25 µl of the Dye Reagent to each well of the microplate containing the standards, controls, and samples.

3. Add 25 µl of the Working Solution to each well of the microplate containing the standards, controls, and samples.

4. Incubate at room temperature for 30 to 60 minutes, protected from light.

5. Read the plate using an absorbance microplate reader at 560 nm or 550 nm.
STANDARD CURVE

Correct for background absorbance. For each point, subtract the negative control from the value derived. Plot the Superoxide Dismutase concentrations vs. the read values to create the standard curve.

Superoxide Dismutase Colorimetric Assay Kit allows for the detection and quantification of endogenous Superoxide Dismutase concentrations within the range of 0.03U/ml - 100U/ml in cell lysates, sera, and plasma.
TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.immunoway.com or contact us at tech@immunoway.com

NOTES