ab65354

Superoxide Dismutase Activity Assay kit (Colorimetric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Superoxide Dismutase Activity in various samples.

This product is for research use only and is not intended for diagnostic use.
# Table of Contents

**INTRODUCTION**
1. BACKGROUND 2
2. ASSAY SUMMARY 3

**GENERAL INFORMATION**
3. PRECAUTIONS 4
4. STORAGE AND STABILITY 4
5. MATERIALS SUPPLIED 5
6. MATERIALS REQUIRED, NOT SUPPLIED 5
7. LIMITATIONS 6
8. TECHNICAL HINTS 7

**ASSAY PREPARATION**
9. REAGENT PREPARATION 8
10. SAMPLE PREPARATION 9

**ASSAY PROCEDURE and DETECTION**
11. ASSAY PROCEDURE and DETECTION 11

**DATA ANALYSIS**
12. CALCULATIONS 12

**RESOURCES**
13. TYPICAL DATA 13
14. QUICK ASSAY PROCEDURE 14
15. TROUBLESHOOTING 15
16. FAQs 17
17. INTERFERENCES 19
18. NOTES 20
1. **BACKGROUND**

Superoxide Dismutase Activity Assay Kit (Colorimetric) (ab65354) is a sensitive kit using WST-1 that produces a water-soluble formazan dye upon reduction with superoxide anion. The rate of the reduction with a superoxide anion is linearly related to the xanthine oxidase (XO) activity, and is inhibited by SOD. Therefore, the inhibition activity of SOD can be determined by a colorimetric method.

Superoxide dismutase (SOD) is one of the most important antioxidative enzymes. It catalyzes the dismutation of the superoxide anion into hydrogen peroxide and molecular oxygen.

**Assay Principle**

\[
\begin{align*}
\text{xanthine} & \overset{XO}{\rightarrow} 2\text{O}_2^- \\
\text{H}_2\text{O}_2 & \overset{SOD}{\rightarrow} \text{O}_2 + \text{H}_2\text{O} \\
\text{O}_2 & \overset{\text{WST-1 formazan}}{\rightarrow} \text{WST-1} \\
\text{WST-1} & \overset{\text{WST-1}}{\rightarrow} \text{WST-1 formazan}
\end{align*}
\]
2. **ASSAY SUMMARY**

<table>
<thead>
<tr>
<th>Step</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard curve preparation (optional)</td>
</tr>
<tr>
<td>Sample preparation</td>
</tr>
<tr>
<td>Add reaction mix and incubate at 37°C for 20 min</td>
</tr>
<tr>
<td>Measure optical density OD450 nm</td>
</tr>
</tbody>
</table>
3. **PRECAUTIONS**

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. **STORAGE AND STABILITY**

Store kit at +4°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months, except Enzyme working solution which is stable for 3 weeks.**
5. **MATERIALS SUPPLIED**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
<th>Storage Condition (After Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WST Solution</td>
<td>1 mL</td>
<td>+4°C</td>
<td>+4°C</td>
</tr>
<tr>
<td>SOD Enzyme Solution</td>
<td>20 µL</td>
<td>+4°C</td>
<td>+4°C</td>
</tr>
<tr>
<td>SOD Assay Buffer</td>
<td>20 mL</td>
<td>+4°C</td>
<td>+4°C</td>
</tr>
<tr>
<td>SOD Dilution BUffer</td>
<td>10 mL</td>
<td>+4°C</td>
<td>+4°C</td>
</tr>
</tbody>
</table>

6. **MATERIALS REQUIRED, NOT SUPPLIED**

These materials are not included in the kit, but will be required to successfully utilize this assay:

- MilliQ water or other type of double distilled water (ddH₂O)
- PBS or 150 mM KCl (if using tissue samples)
- Colorimetric microplate reader – equipped with filter for OD 570 nm
- 96 well plate: clear plates for colorimetric assay
- 0.1M Tris/HCL, pH 7.4 containing 0.5% Triton X-100, 5mM β-ME, 0.1 mg/mL PMSF (if using cell or tissue samples)
- Microcentrifuge
- Pipettes and pipette tips
- Orbital shaker
- Optional: SOD human standard (ab112193) – to be used for standard curve
7. **LIMITATIONS**

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.
8. **TECHNICAL HINTS**

- This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

- Keep enzymes and heat labile components and samples on ice during the assay.

- Make sure all buffers and developing solutions are at room temperature before starting the experiment.

- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.

- Avoid foaming or bubbles when mixing or reconstituting components.

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.

- Ensure plates are properly sealed or covered during incubation steps.

- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.

- Make sure you have the appropriate type of plate for the detection method of choice.

- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.
9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **WST Solution:**

Dilute the 1 mL WST solution with 19 mL of SOD Assay Buffer. Aliquot WST working solution so that you have enough to perform the desired number of assays. Store at +4°C.

9.2 **SOD Enzyme Solution:**

Centrifuge the Enzyme solution for 5 seconds and mix by pipetting. This step is essential as the enzyme has 2 layers and must be mixed well before dilution. Dilute 15 µL with 2.5 mL of Dilution Buffer. This the diluted enzyme solution. This can be stored at +4°C for up to 3 weeks.

9.3 **SOD Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store aliquots at +4°C.

9.4 **SOD Dilution Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot buffer so that you have enough to perform the desired number of assays. Store at +4°C.
10. **SAMPLE PREPARATION**

**General Sample information:**

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

10.1 **Cell (adherent or suspension) samples:**

10.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 10⁶ cells).

10.1.2 Lyse cells in ice cold 0.1M Tris/HCl, pH 7.4 containing 0.5% Triton X-100, 5mM β-ME, 0.1mg/ml PMSF.

10.1.3 Centrifuge at 14,000 x g for 5 minutes at +4°C.

10.1.4 Collect supernatant and transfer to a clean tube.

10.1.5 Keep on ice.

10.2 **Tissue samples:**

10.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg).

10.2.2 Perfuse with PBS or 150 mM KCl, to remove any red blood cells.

10.2.3 Homogenize in ice cold 0.1M Tris/HCl, pH 7.4 containing 0.5% Triton X-100, 5mM β-ME, 0.1mg/ml PMSF.

10.2.4 Centrifuge at 14,000 x g for 5 minutes at +4°C.

10.2.5 Collect supernatant and transfer to clean tube.

10.2.6 Keep on ice.
**NOTE:** If it is desired to measure SOD activity from cytosol and mitochondria separately, cytosol and mitochondria can be separated by using ab65320 (Mitochondria/ Cytosol Fractionation Kit). SOD activity is then measured from the mitochondria and cytosol fractions separately.

**10.3 Blood and plasma samples:**

10.3.1 Collect blood using citrate or EDTA.

10.3.2 Centrifuge at 1,000 x g for 10 minutes at +4°C.

10.3.3 Transfer the plasma layer to a new tube without disturbing the buffy layer.

**NOTE:** Can be stored at -80°C until ready for analysis.

10.3.4 Remove the buffy layer from the red cell pellet.

10.3.5 Re-suspend the erythrocytes in 5X volumes of ice cold distilled water.

10.3.6 Centrifuge at 10,000 x g for 10 minutes (to pellet the erythrocyte membranes).

10.3.7 Store the supernatant at -80°C until ready for analysis.

10.3.8 Plasma can be diluted approx. 3-10X and the red cell lysate can be diluted approx. 100X prior to SOD assay.

**NOTE:** The SOD kit (ab65354) can be used without a standard since it is reporting as % inhibition. The more SOD in a sample the less WST-1 formazan produced (which is dependent on Xanthine Oxidase (XO) activity). You can use purified protein as a standard (SOD full length protein (ab112193)) and then treat the standard just as you would treat a sample.
11. ASSAY PROCEDURE and DETECTION

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all standards, controls and samples in duplicate.

11.1 Set up Reaction wells as per table below:

- Blank 1 = 20 µL H₂O.
- Blank 2 = 20 µL sample.
- Blank 3 = 20 µL H₂O.
- Sample wells = 20 µL samples.

<table>
<thead>
<tr>
<th>Component</th>
<th>Sample (µL)</th>
<th>Blank 1 (µL)</th>
<th>Blank 2 (µL)</th>
<th>Blank 3 (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Solution</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>ddH₂O</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>WST Working Solution</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Enzyme Working Solution</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dilution Buffer</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

**NOTE:** If you are using a SOD standard (not included with the kit), set up wells for it in the same manner as the sample.

11.2 Add 200 µL of WST working solution to each well.
11.3 Add 20 µL of Dilution Buffer to Blank 2 and Blank 3.
11.4 Add 20 µL of Enzyme Working Solution to each sample well and Blank 1.

**NOTE:** Since the superoxide will be released immediately after the addition of Enzyme Working Solution, use a multiple channel pipette to avoid reaction time lag of each well.

11.5 Mix and incubate at 37°C for 20 minutes.
11.6 Measure output (OD450 nm) on a microplate reader.
12. **CALCULATIONS**

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

12.1 Calculate the SOD activity (inhibition rate %) using the following equation:

\[
\text{SOD Activity (inhibition rate %)} = \frac{(A_{\text{blank1}} - A_{\text{blank3}}) - (A_{\text{sample}} - A_{\text{blank2}})}{(A_{\text{blank1}} - A_{\text{blank3}})} \times 100
\]

If Standard Curve is used:

12.2 Average the duplicate reading for each standard and sample.

12.3 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

12.4 Extrapolate sample readings from the standard curve plotted using the following equation:

\[
X = \left( \frac{\text{Corrected absorbance} - (y - \text{intercept})}{\text{Slope}} \right)
\]

12.5 Use sample reading in the equation stated in step 12.1 to workout SOD activity.
Figure 1: Superoxide dismutase measured in biofluids at various dilutions.

Figure 2: Superoxidase dismutase (ab90040) measured showing inhibition rate (%) per concentration (microgram per mL).
14. **QUICK ASSAY PROCEDURE**

*NOTE:* This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when initially performing the assay for the first time.

- Solubilize WST Solution, prepare enzyme mix (aliquot if necessary); get equipment ready.
- (Optional) Prepare standard curve.
- Prepare samples in duplicate.
- Prepare plate as follows:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample Solution</th>
<th>Blank 1</th>
<th>Blank 2</th>
<th>Blank 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>(μL)</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>ddH&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>WST Working Solution</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Enzyme Working Solution</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dilution Buffer</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

- Incubate plate at 37°C for 20 minutes.
- Measure plate at OD450nm for colorimetric assay.
## TROUBLESHOOTING

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay not working</td>
<td>Use of ice-cold buffer</td>
<td>Buffers must be at room temperature</td>
</tr>
<tr>
<td></td>
<td>Plate read at incorrect</td>
<td>Check the wavelength and filter settings of instrument</td>
</tr>
<tr>
<td></td>
<td>wavelength</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Use of inappropriate plate</td>
<td>Colorimetry: Clear plates</td>
</tr>
<tr>
<td></td>
<td>for reader</td>
<td>Fluorescence: Black plates (clear bottom)</td>
</tr>
<tr>
<td>Sample with erratic readings</td>
<td>Samples not deproteinized</td>
<td>Use PCA precipitation protocol for deproteinization</td>
</tr>
<tr>
<td></td>
<td>(if indicated on protocol)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cells/tissue samples not</td>
<td>Use Dounce homogenizer (increase number of strokes);</td>
</tr>
<tr>
<td></td>
<td>homogenized completely</td>
<td>observe for lysis under microscope</td>
</tr>
<tr>
<td></td>
<td>Samples used after multiple</td>
<td>Aliquot and freeze samples if needed to use multiple times</td>
</tr>
<tr>
<td></td>
<td>free/ thaw cycles</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Use of old or inappropriately</td>
<td>Use fresh samples or store at -80°C (after snap freeze in</td>
</tr>
<tr>
<td></td>
<td>stored samples</td>
<td>liquid nitrogen) till use</td>
</tr>
<tr>
<td></td>
<td>Presence of interfering</td>
<td>Check protocol for interfering substances; deproteinize samples</td>
</tr>
<tr>
<td></td>
<td>substance in the sample</td>
<td></td>
</tr>
<tr>
<td>Lower/ Higher readings in</td>
<td>Improperly thawed components</td>
<td>Thaw all components completely and mix gently before use</td>
</tr>
<tr>
<td>samples and Standards</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Allowing reagents to sit</td>
<td>Always thaw and prepare fresh reaction mix before use</td>
</tr>
<tr>
<td></td>
<td>for extended times on ice</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Incorrect incubation times</td>
<td>Verify correct incubation times and temperatures in protocol</td>
</tr>
<tr>
<td></td>
<td>or temperatures</td>
<td></td>
</tr>
<tr>
<td>Problem</td>
<td>Cause</td>
<td>Solution</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>----------------------------------------------</td>
<td>------------------------------------------------------------------</td>
</tr>
<tr>
<td>Standard readings do not follow a linear pattern</td>
<td>Pipetting errors in standard or reaction mix</td>
<td>Avoid pipetting small volumes and prepare a master mix whenever possible</td>
</tr>
<tr>
<td></td>
<td>Air bubbles formed in well</td>
<td>Pipette gently against the wall of the tubes</td>
</tr>
<tr>
<td></td>
<td>Standard stock is at incorrect concentration</td>
<td>Always refer to dilutions on protocol</td>
</tr>
<tr>
<td>Unanticipated results</td>
<td>Measured at incorrect wavelength</td>
<td>Check equipment and filter setting</td>
</tr>
<tr>
<td></td>
<td>Samples contain interfering substances</td>
<td>Troubleshoot if it interferes with the kit</td>
</tr>
<tr>
<td></td>
<td>Sample readings above/below the linear range</td>
<td>Concentrate/Dilute sample so as to be in the linear range</td>
</tr>
</tbody>
</table>
16. **FAQs**

**Is there a formula for transforming relative enzyme activity into absolute enzyme activity?**

Yes, you would need to use a standard to find out the absolute activity. You will have to do a kinetic experiment to get this absolute activity.

How to calculate the activity:
- Plot a standard curve with different concentration of the SOD using exactly this assay protocol.

For all samples, do a kinetic assay:
- Measure OD 450 nm at T1 to read A1
- Measure again at T2 after incubating the reaction at 25°C for 10 - 20 mins (protected from light) (or incubate longer time if the SOD activity is low in sample) to read A2.
- The signal increase is due to SOD, $\Delta A = A2 - A1$

  **NOTE:** It is essential to read $A1$ and $A2$ in the reaction linear range. It will be more accurate if you read the reaction kinetics. Then choose $A1$ and $A2$ in the reaction linear range.

Calculation:
- Subtract 0 standard readings from the standards.
- Plot the SOD standard curve.
- Apply the $\Delta A$ to the standard curve to get B nmol of activity generated between T1 and T2 in the reaction wells.
SOD calculation:

\[
SOD \text{ activity} = \left( \frac{B}{T_2 - T_1} \right) \times V \times D = \frac{\text{nmol}}{\text{min/mL}} = \text{mU/mL}
\]

Where:

- \( B \) = SOD amount from SOD standard curve (in nmol).
- \( T_1 \) = time of the first reading (A1) (in min).
- \( T_2 \) = time of the second reading (A2) (in min).
- \( V \) = sample volume added into the reaction well (in mL)
- \( D \) = Sample dilution factor.
17. **INTERFERENCES**
UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 108008523689 (中國聯通)

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp

Copyright © 2015 Abcam, All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.