ab65354
Superoxide Dismutase Activity Assay kit (Colorimetric)

For the measurement of Superoxide Dismutase Activity in various samples.

View kit datasheet: www.abcam.com/ab65354
(use www.abcam.cn/ab65354 for China, or www.abcam.co.jp/ab65354 for Japan)

This product is for research use only and is not intended for diagnostic use.
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1. Overview

Superoxide Dismutase Activity Assay Kit (Colorimetric) (ab65354) is a robust and sensitive kit for measuring SOD activity in serum, plasma, tissue/cell lysates and other biological fluids.

The assay utilizes a tetrazolium salt WST-1 that produces a water-soluble formazan dye upon reduction with superoxide anion. The rate of WST-1 reduction is linearly related to the inhibition activity of xanthine oxidase (XO) by SOD. SOD catalyzes the dismutation of the superoxide anion into hydrogen peroxide and molecular oxygen resulting in decrease of WST-1 reduction. This inhibition activity of SOD is measured by colorimetric method at OD 450nm.

Assay Principle
Standard Curve Preparation (optional, ab112193)

↓

Sample preparation

↓

Add reaction mix and incubate at 37°C for 20 min

↓

Measure optical density OD 450nm
2. Quick Assay Procedure

△ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Solubilize WST Solution, prepare enzyme mix (aliquot if necessary); get equipment ready.
- Prepare standard curve (Optional).
- Prepare samples in duplicate and in optimal dilutions so that the readings fit within the standard curve readings.
- Prepare plate as follows:

<table>
<thead>
<tr>
<th>Item</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>

- Incubate plate at 37°C for 20 minutes.
- Measure plate at OD 450nm for colorimetric assay.
3. Materials Supplied and Storage

Store kit at +4°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Reconstituted components are stable for 2 months, except Enzyme working solution which is stable for 3 weeks.

Aliquot components in working volumes before storing at the recommended temperature.

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Storage temperature (before prep)</th>
<th>Storage temperature (after prep)</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>
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</tbody>
</table>
4. Materials Required, Not Supplied

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

- PBS or 150 mM KCl (if using tissue samples)
- Dounce homogenizer (if using tissue)
- Microplate reader capable of measuring absorbance at 450 nm
- 96 well plate: clear flat bottom 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)
- Optional: SOD human standard (ab112193) – to be used for standard curve
5. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.
6. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

6.1 WST Solution:
Dilute the 1 mL WST solution with 19 mL of SOD Assay Buffer. Aliquot so that you have enough to perform the desired number of assays.

6.2 SOD Enzyme Solution:
Centrifuge the Enzyme solution for 5 seconds and mix by pipetting, it 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 is the enzyme working solution and can be stored at +4°C for up to 3 weeks.

6.3 SOD Assay Buffer
Ready to use as supplied. Equilibrate to room temperature before use.

6.4 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.
7. 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 for the most reproducible assay. If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them 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. Avoid multiple freeze-thaws.
- The kit is only tested with mammalian samples however as the reactions are species independent therefore, it is expected to work with wide range of sample species like plants, insects, prokaryotes, crustaceans etc. The optimal conditions must be empirically determined by the end user.
- Frozen samples are suitable to use. Avoid multiple freeze-thaw cycles.
- The lower detection limit for this kit is 0.1 U/ml of SOD activity.

△ Note: The kit measures the activity of all isoforms of SOD enzyme and should work with all SODs (Cu/Zn, Mn and FeSOD).

△ Note: For comparing SOD activities between different samples, these can be normalised on protein concentration or cell number. If necessary, the provided assay buffer can be used for diluting samples.

7.1 Cell (adherent or suspension) samples:

1. Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 10⁶ cells).
2. Lyse cells in ice cold 0.1M Tris/HCl, pH 7.4 containing 0.5% Triton X-100, 5mM β-ME, 0.1 mg/ml PMSF.
3. Centrifuge at 14,000 x g for 5 minutes at +4°C.
4. Collect supernatant and transfer to a clean tube.
5. Keep on ice.
7.2 Tissue samples:

1. Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg).
2. Perfuse with PBS or 150 mM KCl, to remove any red blood cells.
3. Homogenize in ice cold 0.1M Tris/HCl, pH 7.4 containing 0.5% Triton X-100, 5mM β-ME, 0.1 mg/ml PMSF.
4. Centrifuge at 14,000 x g for 5 minutes at +4°C.
5. Collect supernatant and transfer to clean tube. The supernatant contains total SOD activity from cytosolic and mitochondrial enzymes.
6. Keep 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, the cytosol and mitochondria can be separated by using ab65320 (Mitochondria/ Cytosol Fractionation Kit). The SOD activity is then measured separately in these fractions.

7.3 Blood and Plasma samples:

1. Collect blood using citrate or EDTA.
2. Centrifuge at 1,000 x g for 10 minutes at +4°C.
3. Transfer the plasma layer to a new tube without disturbing the buffy layer. Plasma can be stored at -80°C until further analysis.
4. Remove the buffy layer from the red cell pellet.
5. Re-suspend the erythrocytes in 5X volumes of ice cold distilled water.
6. Centrifuge at 10,000 x g for 10 minutes (to pellet the erythrocyte membranes).
7. Store the supernatant at -80°C until ready for analysis.
8. Plasma can be diluted 3-10 times and the red cell lysate can be diluted 100 times prior to SOD assay.

△ Note: The kit 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). A purified protein such as SOD full length protein (ab112193) can be
used as standard, and must be treated same way as sample.

**Δ Note:** Plasma prepared using heparin is suitable to use, as the sample gets diluted automatically at various steps so if there will be any effect from heparin, it will be negligible.
8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use, and gently agitate.
- Assay all standards, controls and samples in duplicate.

8.1 Reaction wells set up:

- Blank 1 = 20 µL ddH₂O
- Blank 2 = 20 µL sample
- Blank 3 = 20 µL ddH₂O
- Sample wells = 20 µL samples

<table>
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<td>Dilution Buffer</td>
<td>0</td>
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△ Note: If you are using a SOD standard (not included in the kit), set up wells for it in the same manner as the sample.

1. Add 200 µL of WST working solution to each well.
2. Add 20 µL of Dilution Buffer to Blank 2 and Blank 3.
3. Add 20 µL of Enzyme Working Solution to each sample well and Blank 1.
4. Mix and incubate at 37°C for 20 minutes.
5. Measure output (OD450 nm) on a microplate reader.

△ 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.
9. Data Analysis

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply 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).

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

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

\( A = \text{absorbance} \)

If Standard Curve is used:

9.2 Average the duplicate reading for each standard and sample.

1. 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).
2. Extrapolate sample readings from the standard curve plotted using the following equation:

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

3. Use sample reading in the equation stated in step 9.1 to workout SOD activity.
10. FAQs

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

Yes, by using a standard absolute activity can be measured in a kinetic experiment.

How to calculate the activity:

Plot a standard curve with different concentration of the SOD standard following the assay procedure (section 8).

For all samples, do a kinetic assay:

1. Measure absorbance A1 (OD 450nm) at time T1.
2. Measure absorbance A2 again at time T2 after incubating the reaction at 25°C for 10 - 20 mins (protected from light) (incubate longer time if the SOD activity is low in sample).
3. The increase in signal is due to SOD, calculate $\Delta A = A2 - A1$

△ Note: For accuracy read the reaction kinetics, and choose A1 and A2 in the reaction linear range.

SOD Calculation:

4. Subtract 0 standard readings from the standards.
5. Plot the SOD standard curve.
6. 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:

$$SOD \text{ activity} = \left\{ \frac{B}{(T2 - T1) \ast V} \right\} \ast D = \frac{\text{nmol}}{\text{min} \ast \text{mL}} = \text{mU/mL}$$

Where:
B = SOD amount from SOD standard curve (in nmol).
T1 = time of the first reading (A1) (in min).
T2 = time of the second reading (A2) (in min).
V = sample volume added into the reaction well (in mL).
D = Sample dilution factor.
11. Typical Data

Data provided for demonstration purposes only.

Figure 1. Superoxide dismutase measured in plasma at various dilutions.

Figure 2: Superoxidase dismutase 1 recombinant protein (ab90040) measured showing inhibition rate (%) per concentration (µg/mL).

ab65354 Superoxide Dismutase Activity Assay Kit (Colorimetric)
12. Notes
ab65354 Superoxide Dismutase Activity Assay Kit (Colorimetric)
Technical Support

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