ab65354 Superoxide Dismutase Activity Assay Kit (Colorimetric)

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

For overview, typical data and additional information please visit: www.abcam.com/ab65354
(use abcam.cn/ab65354 for China, or abcam.co.jp/ab65354 for Japan)

Materials Supplied and Storage

Store kit at -20°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. Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
WST Reagent II	1 mL	+4°C	+4°C
SOD Enzyme Solution	1 Vial	-20°C	-20°C
SOD Assay Buffer	20 mL	+4°C	+4°C
SOD Dilution Buffer	10 mL	+4°C	+4°C

Materials Required, Not Supplied:

- PBS or 150 mM KCl (if using tissue samples)
- Dounce homogenizer (if using tissue)
- Microplate reader capable of measuring absorbance at 440 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

1. Reagent Preparation:

Briefly centrifuge small vials at low speed prior to opening.

1.1 WST Reagent II: Prior to use, thaw to room temperature and prepare a WST working solution by diluting the WST Reagent II at a 1:20 ratio with SOD Assay Buffer (for an entire assay plate, mix 1 mL WST Reagent II with 19 mL SOD Assay Buffer). Make/aliquot so that you have enough to perform the desired number of assays. Diluted WST working solution may be stored for up to 2 months at +4°C, protected from light.

1.2 SOD Enzyme Solution: Reconstitute lyophilized enzyme with 110 µl ddH_2O. Divide into aliquots and store at -20°C. Avoid repeated freeze/thaw cycles. Use within 2 months.

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

1.4 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.

2. Sample Preparation

- 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, 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.
- The lower detection limit for this kit is 0.1 U/ml of SOD activity.

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

Δ 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.

2.1 Cell (adherent or suspension) samples:

1. Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 10^6 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.

2.2 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.

   Δ Note: RIPA buffer (without SDS) supplemented with PMSF protease inhibitors can also be used as lysis buffer.

   Δ 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.

2.3 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.

Δ 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 in the 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.

3. Assay Procedure:

Prior to performing assay, prepare Enzyme Working Solution by diluting the reconstituted SOD Enzyme Solution at a 1:20 ratio using SOD Dilution Buffer. Prepare 20 µl of Enzyme Working Solution for each Sample and Blank 1 reaction to be performed (for example, for 10 wells, mix 10 µl of SOD Enzyme Solution stock with 190 µl SOD Dilution Buffer). Equilibrate all other materials and prepared reagents to room temperature just prior to use, and gently agitate. Assay all standards, controls and samples in duplicate.

3.1 Reaction wells set up:
- Blank 1 = 20 µL ddH_2O
- Blank 2 = 20 µL sample
- Blank 3 = 20 µL ddH_2O
- Sample wells = 20 µL samples

Component	Sample (µL)	Blank 1 (µL)	Blank 2 (µL)	Blank 3 (µL)
Sample Solution	20	0	20	0
ddH_2O	0	20	0	20
WST Working Solution	200	200	200	200
Enzyme Working Solution	20	20	0	0
SOD Dilution Buffer	0	0	20	20

Δ 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.
Δ Note: For specific activity of SOD, it is recommended to do a series 1/10 dilutions and obtain IC50 (µg). SOD Activity (U/mg) = 1000/IC50 (µg)
ΔNote: Blank 2 is paired with each sample, Blank 1 and Blank 3 are run with each experiment.
Unit Definition: One Unit is defined as the amount of SOD that inhibits the XO activity by 50% (IC50 (µg)) under the assay conditions.
3.2 Add 200 µL of WST working solution to each well.
3.3 Add 20 µL of Dilution Buffer to Blank 2 and Blank 3.
3.4 Add 20 µL of Enzyme Working Solution to each sample well and Blank 1.
3.5 Mix and incubate at 37°C for 20 minutes.
3.6 Measure output (OD440 nm) on a microplate reader.
Δ Note: Since the superoxide will be released immediately after the addition of Enzyme Working Solution, use a multichannel pipette to avoid reaction time lag between wells.
Δ Note: Once prepared, the Enzyme Working Solution should be used promptly. Do not store or freeze Enzyme Working Solution.

4. 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).

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

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

A = absorbance (OD) at 440 nm

If Standard Curve is used:

4.2 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:

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

3. Use sample reading in the equation stated in step 5.1 to workout SOD activity.

Technical Support:

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