MANUAL

RayBio® Superoxide Dismutase (SOD) Activity Assay Kit (Colorimetric)

Catalog #: MA-SOD

RayBiotech
Empowering your proteomics

ISO 13485:2016

Introduction

Superoxide Dismutases (SODs) are metalloenzymes crucial for cellular antioxidant defense, catalyzing the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide. Three characterized types include copper/zinc (Cu/Zn), manganese (Mn), and iron (Fe) SODs, with widespread distribution in plants and animals, particularly in organs such as the brain, liver, heart, erythrocytes, and kidney. Maintaining appropriate SOD levels in cellular and extracellular environments is crucial for the prevention of diseases associated with oxidative stress, including neurodegenerative disorders like Alzheimer's, Parkinson's, and Huntington's diseases.

RayBio® Superoxide Dismutase (SOD) Activity Assay Kit provides a simple, reproducible, and sensitive tool for measuring SOD activity in plasma, serum, cell lysates and other biological samples. In this assay, superoxide anions, generated through xanthine conversion to uric acid and hydrogen peroxide by xanthine oxidase (XO), reduce WST-1 to water-soluble formazans. The absorbance at 450nm measures the formazans, and SODs modulate the reduction reaction rate by diminishing superoxide anion concentrations. Consequently, the percentage of inhibition in the reduction reaction serves as a quantifiable indicator of SOD activity.

Storage

The entire kit may be stored at < -20 °C for up to 6 months from the date of shipment. For prepared reagent storage, see table below.

Component	Size / Description	Storage After Preparation
Microplate (Item A)	A 96-well (12 strips x 8 wells) plate	RT*
Sample Buffer	10 ml	2–8 °C, 4 weeks
Assay Buffer	25 ml	2–8 °C, 4 weeks
Substrate Concentrate	1 amber vial (Lyophilized)	-20 °C. 4 weeks
Cofactor	1 amber vial (Lyophilized)	-20 °C. 4 weeks
SOD Standard	1 vial (Lyophilized)	Aliquot and store at -80 °C, 3 months, do not store the diluted standard.
XO Concentrate	2 amber vials (Lyophilized)	Do not store and re-use

RT = room temperature
**Return unused wells to the pouch containing desiccant pack, reseal along entire edge.*

Additional Materials Required

1. Microplate reader capable of measuring absorbance at 450 nm
2. Precision pipettes to deliver 2 µl to 1 ml volumes
3. Multi-channel pipettes to deliver 20 µl to 200 µl volumes
4. Tubes to prepare sample dilutions
5. Distilled or deionized water
6. 50 ml conical tubes

Sample Tips and General Considerations

NOTE: Optimal methods of sample preparation will need to be determined by each researcher empirically based on researched literature and knowledge of the samples.

- If not using fresh samples, freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot samples prior to initial storage.
- It is strongly recommended to add a protease inhibitor cocktail to cell and tissue lysate samples.
- Avoid sonication of 1 ml or less as this can quickly heat and denature proteins.
- Most samples will not need to be concentrated. If concentration is required, a spin column concentrator with a chilled centrifuge is recommended.

1. Cell lysates can be prepared as follows:

For attached cells, remove supernatant from cell culture, wash cells twice with cold 1X PBS (for suspension cells, pellet the cells by spinning down the cells at 1,000 x g for 10 min) making sure to remove any remaining PBS before adding lysis buffer. Solubilize the cells at $2x10^7$ cells/ml in lysis buffer containing protease inhibitors. Pipette up and down to resuspend cells and rock the lysates gently at 2–8 °C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 14,000 x g for 10 minutes.
It is recommended that sample protein concentrations should be determined using a total protein assay. Lysates should be used immediately or aliquot and stored at -70 °C. Thawed lysates should be kept on ice prior to use.
General tips for preparing lysate samples can be viewed on the online Resources page of the website: https://www.raybiotech.com/tips-on-sample-preparation/

2. Plasma samples:

Collect blood with an anticoagulant such as citrate, EDTA or oxalate and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer. Sample should be tested immediately or frozen at -80°C for storage. Dilute plasma samples at least 1:5 in Sample Buffer.

3. Serum samples:

Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C for storage. Dilute serum samples at least 1:5 in Sample Buffer.

Note: Use all samples within 2 hours of dilution. If the calculated Superoxide Dismutase activity of the sample is higher than 4 U/mL, dilute the sample in sample buffer and repeat the assay.

Reagent Preparation

REAGENT	PREPARATION
Microplate (Item A)	No preparation
Sample Buffer	
Assay Buffer	
SOD Standard	See “Standard Preparation” section.
Substrate Concentrate	Reconstitute with 102 µL DiH_2O. Store at -20 °C after preparation
Cofactor	Reconstitute with 22 µL DiH_2O. Store at -20 °C after preparation
Substrate Working Solution	Add 50 µL reconstituted Substrate Concentrate and 10 µL reconstituted Cofactor into 10.5 mL Assay Buffer to make Substrate Working Solution. This is enough for half the plate. The solution is stable for 1 day at 2-8°C in the dark.
XO Concentrate	Reconstitute with 1 ml Sample Buffer for each vial.
XO Working Solution	Add 1 mL reconstituted XO Concentrate into 1.5 mL Sample Buffer to make XO Working Solution. This is enough for half the plate. The solution is stable for 1 day at 2-8°C in the dark.

Standard Preparation

To prepare a dilution series of standard in the concentration range of 0 U/mL – 4 U/mL (see Table below), *Note:* Use the standards within 2 hours of preparation.

1. Label 8 microtubes #1 through 8 which with the following concentrations: 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, 0 U/mL.
2. Pipette 498 µL Sample Buffer into labeled tube #1
3. Pipette 100 µL Sample Buffer into labeled tubes #2 – tube #8.
4. To make 1,000 U/mL SOD Standard Stock: Briefly spin down the SOD Standard, pipette 20 µL of Sample Buffer into the tube, vortex and quick spin down. Pipette 3.8 µL of reconstituted SOD Standard into 96.2 µL Sample Buffer, mix thoroughly to get 1,000 U/mL SOD Standard Stock.
5. Pipette 2 µL 1,000 U/mL SOD Standard Stock into tube #1, mix thoroughly to make 4 U/mL SOD Standard.
6. To make the 2 U/mL standard, pipette 100 µL tube #1 into the tube labeled #2. Mix thoroughly.
7. Repeat this step with each successive concentration, preparing a dilution series as shown in the Table below. Each time, use 100 µl of the prior concentration until the 0.0625 U/mL is reached. Mix each tube thoroughly before the next transfer. Standards should be prepared freshly, mixed thoroughly, and used immediately.

Labeled Tubes	SOD Standard (µL)	Sample Buffer (µL)	Standard Activity (U/mL)
1	2 µL of 1,000 U/mL Stock	498 µL	4
2	100 µL of Tube #1	100 µL	2
3	100 µL of Tube #2	100 µL	1
4	100 µL of Tube #3	100 µL	0.5
5	100 µL of Tube #4	100 µL	0.25
6	100 µL of Tube #5	100 µL	0.125
7	100 µL of Tube #6	100 µL	0.0625
8	0 µL	100 µL	0

Assay Procedure

Each Superoxide Dismutase standard and samples should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add 20 µL of the diluted SOD standards or unknown samples to the 96-well Microplate.
2. Add 200 µL Substrate Working Solution to each well (See “Reagent Preparation”). Mix thoroughly. Read and record absorbance at 450nm (A1 Reading).
3. Initiate the reaction by adding 30 µL XO Working Solution (See “Reagent Preparation”) into each well and mix thoroughly.
4. Incubate for 30 minutes at room temperature.
5. Read and record absorbance at 450nm (A2 Reading).

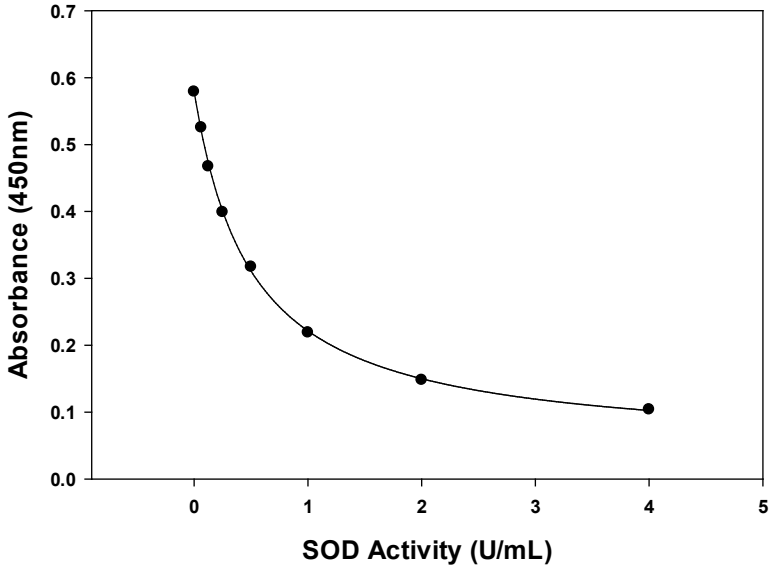
Calculation of Results

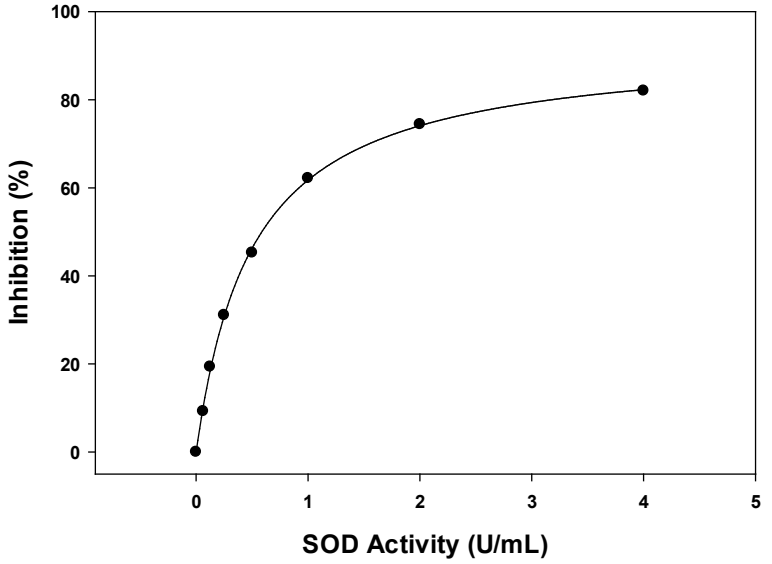
1. Calculate the average absorbance values for each set of standards and samples.
2. Calculate the corrected absorbance for each standard and sample: ΔA = A2 – A1
3. Plot the standard curve using SigmaPlot software (or other software which can perform four-parameter logistic regression models), with standard activity on the x-axis and corrected absorbance (ΔA) on the y-axis. Draw the best-fit curve through the standard points.
4. Use the mean absorbance value for each sample to determine the corresponding activity from the standard curve.
5. Inhibition percentage can be calculated using the function below:

$$\text{Inhibition \%} = \frac{\Delta A_{\text{Blank}} - \Delta A_{\text{Sample}}}{\Delta A_{\text{Blank}}} \times 100$$

Note: If the calculated Superoxide Dismutase activity of the sample is higher than 4 U/mL, dilute the sample in sample buffer and repeat the assay.

A. Typical Data





These standard curves are for demonstration only. A standard curve must be run with each assay.

B. Reproducibility

Intra-assay Precision (Precision within an assay):
To assess intra-assay precision, 16 wells per sample (total of 4 samples) were tested on a single plate. The intra-assay coefficient of variation was found to be 7.4%.

Inter-assay Precision (Precision between assays):
To assess inter-assay precision, 4 samples were tested in separate assays (n=4). The inter-assay coefficient of variation was found to be 8.7%.

This product is for research use only.