Superoxide Dismutase Protocol

Version: 1
Replaced by version
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Summary:

Significant amounts of superoxide dismutase (SOD) in cellular and extracellular environments are crucial for the prevention of diseases linked to oxidative stress. Mutations in SOD account for approximately 20% of familial amyotrophic lateral sclerosis (ALS) cases. SOD also appears to be important in the prevention of other neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's Diseases. The reaction catalyzed by SOD is extremely fast, having a turnover of 2 x 10^6 M-1sec-1 and the presence of sufficient amounts of the enzyme in cells and tissues typically keeps the concentration of superoxide very low. Quantification of SOD activity is therefore essential in order to fully characterize the antioxidant capabilities of a biological system. The Cayman Chemical SOD Assay kit is a fast and reliable assay for the measurement of SOD activity from plasma, serum, tissue homogenates, and cell lysates. SOD activity is assessed by measuring the dismutation of superoxide radicals generated by xanthine oxidase and hypoxanthine in a convenient 96 well format. A key feature of the kit is the inclusion of a quality-controlled SOD standard. The standard curve generated using this enzyme provides a means to accurately quantify the activity of all three types of SOD (Cu/Zn-, Mn-, and Fe-SOD). Each kit contains sufficient reagents to assay 41 samples in duplicate and includes assay buffer, sample buffer, radical detector, SOD (standard), xanthine oxidase, a 96 well plate, and complete instructions.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Assay Kit	Cayman	706002
Assay Buffer		
Sample Buffer		
Radical Detector		
Standard		
Xanthine Oxidase		

Protocol:

1. **SOD Standard Wells** - add 200 µl of the diluted Radical Detector and 10 µl of Standard (tubes A-G) per well in the designated wells on the plate (see sample plate format, Figure 2, page 11).
2. **Sample Wells** - add 200 µl of the diluted Radical Detector and 10 µl of sample to the wells. *NOTE: If using an inhibitor, add 190 µl of the diluted Radical Detector, 10 µl of inhibitor, and 10 µl of sample to the wells. The amount of sample added to the well should always be 10 µl. Samples should be diluted with Sample Buffer (dilute) or concentrated with an Amicon centrifuge concentrator with a molecular weight cut-off of 10,000 to bring the enzymatic activity to fall within the standard curve range.*
3. Initiate the reactions by adding 20 µl of diluted Xanthine Oxidase to all the wells you are using. Make sure to note the precise time you started and add the Xanthine Oxidase as quickly as possible. *NOTE: If assaying sample backgrounds, add 20 µl of Sample Buffer instead of Xanthine Oxidase.*
4. Carefully shake the 96-well plate for a few seconds to mix. Cover with the plate cover.
5. Incubate the plate on a shaker for 20 minutes at room temperature. Read the absorbance at 440-460 nm using a plate reader.

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1. Calculate the average absorbance of each standard and sample. If assayed, subtract sample background absorbance from the sample.
2. Divide standard A's absorbance by itself and divide standard A's absorbance by all the other standards and samples absorbances to yield the linearized rate (LR) (*i.e.*, LR for Std A = Abs Std A/Abs Std A; LR for Std B = Abs Std A/Abs Std B).
3. Plot the linearized SOD standard rate (LR) (from step 2 above) as a function of final SOD Activity (U/ml) from Table 1. See Figure 3 (on page 17) for a typical standard curve.
4. Calculate the SOD activity of the samples using the equation obtained from the linear regression of the standard curve substituting the linearized rate (LR) for each sample. One unit is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. SOD activity is standardized using the cytochrome c and xanthine oxidase coupled assay

$$\text{SOD (U/ml)} = \left[\left(\frac{\text{sample LR - y-intercept}}{\text{slope}}\right) \times \frac{0.23\ \text{ml}}{0.01\ \text{ml}}\right] \times \text{sample dilution}$$