Superoxide Dismutase Kit

Catalog Number: 7500-100-K

Reagent kit for the analysis of Superoxide Dismutase in cell extracts.

Sufficient reagents for 100 experimental tests, 50 negative controls, and 50 positive controls.

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTRODUCTION

Superoxide Dismutase (SOD) catalyzes the dismutation of the superoxide radical (O_2^-) into hydrogen peroxide (H_2O_2) and elemental oxygen (O_2) (Figure 1) and as such provides an important defense against the toxicity of the superoxide radical. In fact, overexpression of SOD protects murine fibrosarcoma cells from apoptosis and promotes cell differentiation (1). SOD also inhibits adriamycin-induced apoptosis in murine peritoneal macrophages (2). In the assay, superoxide ions (O_2^-), generated by xanthine oxidase (XOD) conversion of xanthine to uric acid and hydrogen peroxide (Figure 1), converts NBT to NBT-diformazan, which absorbs light at 560 nm. SOD reduces the superoxide ion concentration and thereby lowers the rate of NBT-diformazan formation. The extent of reduction in the appearance of NBT-diformazan is a measure of SOD activity present in an experimental sample. The assay is free of interference by other catalytic activities and is ideal for assaying SOD in mammalian cell lysates. The kit contains the lysis buffer and the reagents needed for 100 experimental tests, 50 negative controls and 50 positive controls. Additionally, this system is not greatly disturbed by trace metals. Each assay requires approximately 5 minutes and after a simple calculation, the percent inhibition of the formation of NBT-diformazan by SOD is converted to the relative activity of the sample.

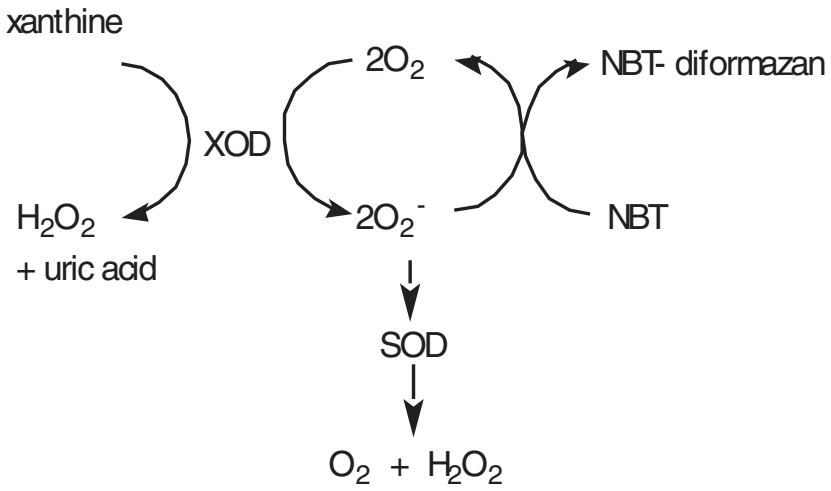

Figure 1: Relationship between XOD, SOD, substrates, products, and the superoxide radical.

FEATURES

- Suitable for mammalian cells.
- Each sample takes only 5 minutes.
- Contains SOD for 50 positive controls.
- Suitable for the assay of Mn-SOD, Fe-SOD, and Cu/Zn-SOD.

PRECAUTIONS AND LIMITATIONS

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The physical, chemical and toxicological properties of these products may not yet have been fully investigated. Therefore, R&D Systems recommends the use of gloves, protective clothing, and eye protection while using these chemical reagents. R&D Systems assumes no liability for damage resulting from handling or contact with these products. MSDS are available upon request.

REAGENTS PROVIDED

Component	Part Number	Quantity	Storage
SOD (1 unit/μL)	7500-100-01	200 μL	2 - 8° C
25X SOD Reaction Buffer	7500-100-02	12 mL	2 - 8° C
Xanthine Solution	7500-100-03	1.5 mL	2 - 8° C
NBT Solution	7500-100-04	6 mL	2 - 8° C
XOD Solution	7500-100-05	2 mL	2 - 8° C
20X Cell Lysis Solution	7500-100-06	12 mL	2 - 8° C

ADDITIONAL MATERIALS REQUIRED

- High quality, double-distilled water
- Spectrophotometer to read absorbance at 550 nm
- Cuvettes (disposable or quartz, with at least a 1.5 mL volume)
- Pipettes and pipette tips
- Pipette aid
- Pasteur pipette and bulb
- Centrifuge
- Timer to read at 30 second intervals

REAGENT PREPARATION

Prior to each experiment, prepare the necessary amount of 1X Cell Lysis Solution by diluting the 20X Cell Lysis Solution with distilled water (dH_2O). All other reagents are ready for use. Store all components at 2 - 8° C until needed and take measures to prevent reagent contamination.

ASSAY PROTOCOL

A. Cell Lysate Preparation

1. Detach adherent cells by gentle trypsinization. Count the cells and centrifuge for 10 minutes at 250 x g at 2 - 8° C. Wash the cells once with cold 1X PBS.

   Note: For trypsinization of adherent cells, use 0.5% (w/v) trypsin, 0.2% (w/v) EDTA according to the procedure in Ausubel, F.M. *et al*. (1988) Appendix A. in *Current Protocols in Molecular Biology*, p. A.3F.5.

2. Suspend the pellet with 500 µL of 1X Cell Lysis Solution per 1 - 5 x 10^6 cells. Mix thoroughly by repeated pipetting.
3. Transfer the suspension to a 1.5 mL tube and centrifuge for 5 minutes at 12,000 - 14,000 x g at 2 - 8° C. Place the supernate into a clean 1.5 mL tube. Store on ice and assay for SOD immediately or store at ≤ -80° C.

B. Tissue Lysate Preparation

1. Liver and other tissues may be lysed and processed in Isotonic Buffer (10 mM Tric-HCl (pH 7.4), 200 mM mannitol, 50 mM sucrose, 1 mM EDTA) (7). Briefly perfuse the organs with PBS containing 0.16 mg/mL of heparin. Weigh, mince, and homogenize the tissue in ice-cold Isotonic Buffer (10 mL/g of tissue) by means of a Teflon pestle in a Potter-Elvejhem homogenizer. Isolate the crude nuclear fraction by centrifugation at 1000 x g at 2 - 8° C for 10 minutes, heavy mitochondria at 3000 x g at 2 - 8° C for 10 minutes, light mitochondria at 20,000 x g at 2 - 8° C for 20 minutes, and microsomes at 144,000 x g at 2 - 8° C for 90 minutes. The final supernate is the cytosolic fraction. Store processed samples on ice and assay for SOD immediately or aliquot and store at ≤ -80° C.

C. Erythrocyte Lysate Preparation

1. Collect blood with heparin or EDTA as an anticoagulant and centrifuge at 2500 x g at 2 - 8° C for 5 - 10 minutes. Remove the plasma supernate and the upper layer of the red blood cell pellet which contains the buffy coat. Suspend the erythrocytes in 4 volumes of ice-cold water and mix thoroughly. Allow 5 minutes for lysis to occur. Assay for SOD immediately or aliquot and store at ≤ -80° C.

D. Differentiation of Mn/Fe-SOD from Cu/Zn-SOD

1. Mn- and Fe-SOD can be inactivated by adding 400 µL or 800 µL of ice-cold chloroform/ethanol (37.5/62.5 (v/v)) to 250 µL of erythrocyte lysate or 500 µL of tissue lysate, respectively, shaking for 30 seconds and centrifuging at 2500 x g for 10 minutes (8). Assay the aqueous phase for Cu/Zn-SOD immediately or aliquot and store at ≤ -80° C.
2. The addition of cyanide ion to a final concentration of 2 mM inhibits Cu/Zn-SOD by over 90%. Mn-SOD is unaffected by cyanide.

E. SOD Assay Procedure

1. The assay is performed at room temperature. All components except cell lysates should be brought to room temperature before use. The total reaction volume is 1.5 mL. The volume of the reagent components is 107.5 μL. Therefore, the volume of deionized or distilled water required equals 1500 μL - 107.5 μL - sample volume (μL).
2. Briefly vortex each reagent immediately before use. To a disposable cuvette, add the following components in the following order:

dH_2O	From Step E-1
25X Reaction Buffer	60 μL
Xanthine Solution	7.5 μL

3. Mix thoroughly by repeated pipetting with a clean Pasteur pipette.
4. Add 30 μL of NBT Solution and repeat Step E-3.
5. Add cell lysate and repeat Step E-3.
6. Place the cuvette into a visible spectrophotometer at a wavelength of 550 nm and set the absorbance reading to zero.
7. Briefly vortex the XOD Solution and add 10 μL to the cuvette. Quickly repeat Step E-3.
8. Immediately place the cuvette into the spectrophotometer, start a timer, and record the absorbance reading every 30 seconds for a period of 5 minutes. The first time point will be at 30 seconds (0:30) and the final point will be at 5 minutes and 30 seconds (5:30).

F. Controls

1. Negative Control

   A negative control must be run. This control includes all components except SOD or cell lysate. In this case, the increase in absorbance due to the generation of superoxide radical proceeds maximally.

2. Positive Control

   This kit contains sufficient SOD for generating 10 standard curves or 50 individual positive controls. The SOD has a concentration of 1 unit/μL (1 unit is that amount of SOD which inhibits the rate of increase in absorbance due to NBT-diformazan formation by 50%). A typical standard curve would include the following SOD concentrations: 0.1 unit, 1 unit, 2 units, 5 units, and 10 units. For the 0.1 unit point, dilute 1 μL of SOD to 10 μL with 1X Reaction Buffer and use 1 μL. Add 1 μL, 2 μL, 5 μL, and 10 μL of undiluted SOD for the 1 unit, 2 unit, 5 unit, and 10 unit activity points, respectively. Briefly vortex the SOD immediately before use.

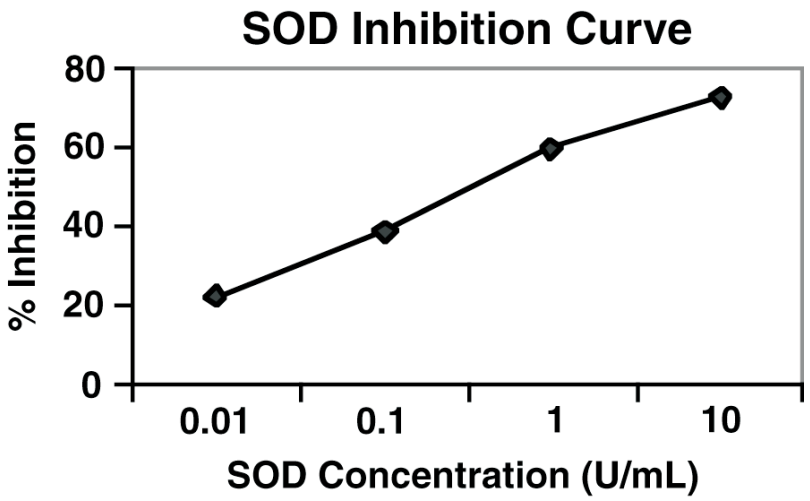

Figure 2: Plot of SOD concentration vs. % Inhibition of the rate of increase of absorbance at 550 nm due to the reduction of NBT to NBT-diformazan by the superoxide radical (O_2^-).

DATA INTERPRETATION

1. Determine the rate of increase in absorbance units (A) per minute for the negative control and for the test sample(s).

$$\frac{A_{550\ nm} \text{ at } 5{:}30 - A_{550nm} \text{ at } 0{:}30}{5 \text{ minutes}} = \Delta A_{550\ nm}/\text{minute}$$

2. Determine the % inhibition for the test sample(s).

$$\frac{[(\Delta A_{550\ nm}/\text{minute}^{(-)\ \text{control}} - (\Delta A_{550nm}/\text{minute})^{\text{Test}}]}{(\Delta A_{550\ nm}/\text{minute})^{(-)\ \text{control}}} \times 100 = \%\ \text{inhibition}$$

3. Calculate the SOD Activity

   One unit of SOD inhibits the rate of increase in absorbance at 550 nm by 50% under the conditions of the assay. The percent inhibition of the test sample correlates with SOD activity using a SOD standard curve.

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