SUPEROXIDE DISMUTASE (SOD)

[EC 1.15.1.1]

from *Bacillus stearothermophilus*

\[ O_2^- + O_2^- + 2H^+ \leftrightarrow O_2 + H_2O_2 \]

**SPECIFICATION**

- **State**: Lyophilized
- **Specific activity**: more than 9,000 U/mg protein
- **Contaminants**: (as SOD activity = 100 %)
  - Catalase: < 0.01 %

**PROPERTIES**

- **Molecular weight**: ca. 50,000
- **Subunit molecular weight**: ca. 25,000
- **Metal content**: 1.5 g atoms of Mn per mole of enzyme
- **Optimum pH**: 9.5 (Fig. 1)
- **pH stability**: 6.0 - 9.0 (Fig. 2)
- **Isoelectric point**: 4.5
- **Thermal stability**: No detectable decrease in activity up to 60 °C. (Fig. 3, 4)

**STORAGE**

Stable at -20 °C for at least one year

**APPLICATION**

The enzyme is useful for medicine, cosmetic material and nutrition or antioxidant.
**ASSAY**

**Principle**
To determine the enzyme activity of cytochrome c reduction is measured by the following reactions.

\[
\text{Xanthine} + O_2 \xrightarrow{\text{Xanthine oxidase}} \text{Urate} + O_2^- + H_2O_2
\]

\[
O_2^- \xrightarrow{\text{SOD}} \text{O}_2 + H_2O_2
\]

\[
\text{cytochrome c} \xrightarrow{\text{reduction}} \text{cytochrome c (red.)}
\]

**Unit Definition**
One unit of activity is defined as the amount of SOD required to inhibit the rate of reduction of cytochrome C by 50 % at 30 °C.

**Solutions**

I. Buffer solution ; 75 mM Potassium phosphate buffer, pH 7.8
II. Xanthine solution ; 0.75 mM (0.010 g xanthine/50 mL N/250 NaOH)
III. Cytochrome c solution ; 0.15 mM (0.019 g cytochrome c/10 mL distilled water, Sigma-Aldrich Co., No. C-2506, from horse heart)
IV. EDTA solution ; 1.5 mM (0.028 g EDTA disodium salt·2H_2O/50 mL distilled water)
V. Xanthine oxidase (XOD) ; (from buttermilk, Sigma-Aldrich Co., No. X-1875) suspension in 2.3 M (NH_4)_2SO_4 solution is diluted to 0.04 U/mL with distilled water. (prepare freshly)

**Preparation of Enzyme Solution**
Dissolve the lyophilized enzyme with distilled water and dilute to approx. 600 U/mL with 50 mM potassium phosphate buffer, pH 7.5.

**Procedure**
1. Prepare the following reaction mixture and pipette 2.80 mL of reaction mixture and 0.005 mL of enzyme solution into a cuvette.
   - Solution I 22.00mL
   - Solution II 2.00mL
   - Solution III 2.00mL
   - Solution IV 2.00mL
2. Incubate at 30 °C for about 3 minutes.
3. Add 0.20 mL of Solution V into the cuvette and mix.
4. Read absorbance change at 550 nm per minute for the linear portion of curve (ΔAbs·test)*.
5. Add 0.005 mL of Solution I in place of enzyme solution and measure the same above 4 (ΔAbs·blank).

* Dilute enzyme solution with 50 mM potassium phosphate buffer, pH 7.5, because the decrease in the initial rate should not fall outside the range of 40 to 60 % for the results to be valid.

**Calculation**

Volume activity (U/mL) = \[ \frac{(\Delta\text{Abs·blank})}{(\Delta\text{Abs·test})} - 1 \] \times \frac{601}{X \text{ d.f.}}

Specific activity (U/mg protein) = \[ \frac{\text{Volume activity (U/mL)}}{\text{protein concentration (mg/mL)}} \]

d.f. ; dilution factor
* Protein concentration ; determined by Bradford's method

**REFERENCE**
Fig. 1  pH profile

△ acetate,  ○ phosphate,

Fig. 2  pH stability

treated for 24 hr at 4 °C in the

Fig. 3  Thermal stability

treated for 15 min in 0.1 M potassium phosphate buffer, pH 7.5

Fig. 4  Thermal stability

treated in 0.1 M potassium phosphate buffer, pH 7.5