

## ● TOYOBO ENZYMES ●

(Diagnostic Reagent Grade)

# SUPEROXIDE DISMUTASE

*from Bovine erythrocyte*

Superoxide:superoxide oxidoreductase (EC 1.15.1.1)

**PREPARATION and SPECIFICATION**

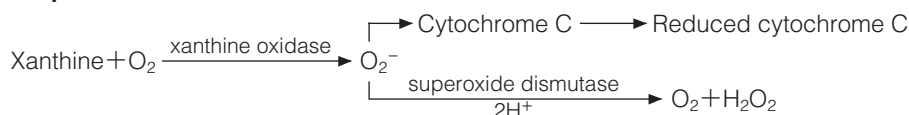
Appearance	: Bluish green amorphous powder, lyophilized
Activity	: Grade III 3,000U/mg-solid or more
Contaminant	: Catalase $\leq 1.0 \times 10^{-2}\%$

**PROPERTIES**

Stability	: Store at $-20^\circ\text{C}$ (Fig.1,2) (A decrease in activity of ca.10% may occur within 6 months)
Molecular weight	: 32,000 <sup>1)</sup>
Isoelectric point	: 4.95 <sup>2)</sup>
Structure	: 2 subunits per mole of enzyme (Each one mole of copper and zinc is bound to each subunit)
Inhibitors	: Cyanide <sup>4)</sup> , diethyldithiocarbamate <sup>5)</sup>
Optimum pH	: 9.0 (Fig.3)
Optimum temperature	: $30^\circ\text{C}$ (Fig.4)
pH Stability	: pH 7.0–8.5 ( $25^\circ\text{C}$ , 20hr) (Fig.5)
Thermal stability	: below $70^\circ\text{C}$ (pH 7.0, 30min) (Fig.6)

## ASSAY

### Principle:



The appearance of reduced cytochrome C is measured at 550nm by spectrophotometry.

### Unit definition:

One unit causes half a maximum inhibition of cytochrome C reduction under the conditions described below.

### Method:

#### Reagents

- A. K-Phosphate buffer, pH 7.8 : 75mM
- B. EDTA solution : 1.5mM Ethylenediaminetetraacetate · Na<sub>2</sub>
- C. Xanthine solution : 0.75mM (Dissolved in 0.004N NaOH solution)(Should be prepared fresh)
- D. Xanthine oxidase solution : 0.04U/ml [Dilute xanthine oxidase (ammonium sulfate suspension, ca.4U/ml) to 0.04U/ml with H<sub>2</sub>O] (Should be prepared fresh)
- E. Cytochrome C solution : 0.15mM (from horse heart)(Should be prepared fresh)
- F. Enzyme diluent : 10mM K-Phosphate buffer,pH 7.8

#### Procedure

1. Prepare the following reaction mixture in a cuvette (d= 1.0cm)

and equilibrate at 25°C for about 5 minutes.

- |        |                       |     |
|--------|-----------------------|-----|
| 2.0 ml | Buffer solution       | (A) |
| 0.20ml | EDTA solution         | (B) |
| 0.20ml | Xanthine solution     | (C) |
| 0.20ml | Cytochrome C solution | (E) |
| 0.20ml | Enzyme solution*      | (F) |

Concentration in assay mixture		
K-Phosphate buffer	57	mM
Xanthine	50	μ M
Cytochrome C	10	mM
EDTA	0.10	mM
Xanthine oxidase	ca.0.90	mU/ml

2. Add 0.2ml of xanthine oxidase solution (D) and mix by gentle inversion.
3. Record the increase in optical density at 550nm against water for 2 to 3 minutes in a spectrophotometer thermostated at 25°C, and calculate the ΔOD per minute from the initial linear portion of the curve (ΔOD test).

At the same time, measure the blank rate (ΔOD blank) by using the same method as the test except that the enzyme diluent (F) is added instead of the enzyme solution.

- \* Dissolve the enzyme preparation in ice-cold enzyme diluent (F) and dilute to 0.5–2.0U/ml with the same buffer and store on ice.

#### Calculation

Activity can be calculated by using the following formula :

$$\text{Volume activity (U/ml)} = \left( \frac{\Delta \text{OD blank}}{\Delta \text{OD test}} - 1 \right) \times \frac{1}{V_s} \times df$$

$$\text{Weight activity (U/mg)} = (\text{U/ml}) \times 1/C$$

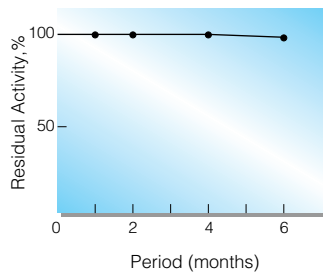
V<sub>s</sub> : Sample volume (0.2ml)

df : Dilution factor

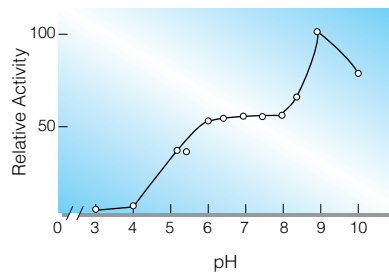
C : Enzyme concentration in dissolution (c mg/ml)

## REFERENCES

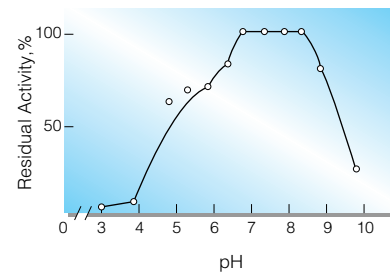
- 1) J.M.McCord and I.Fridovich; *J.Biol.Chem*, **244**, 6049 (1969)
- 2) J.Bannister, W.Bannister and E.Wood; *Eur.J.Biochem.*, **18**, 178 (1971)
- 3) I.Fridovich; *Advan.Enzymol.*, **41**, 35 (1974)
- 4) C.O.Beauchamp and I.Fridovich; *Biochim.Biophys.Acta.*, **317**, 50 (1973)
- 5) R.E.Heikkila, F.S.Cabbat and G.Cohen; *J.Biol.Chem.*, **251**, 2182 (1976)



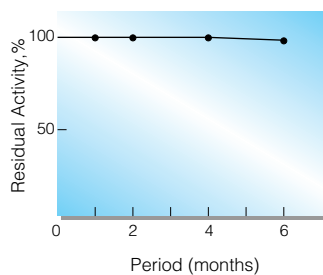
**Fig.1. Stability (Powder form at 5°C)**  
[ kept under dry conditions ]



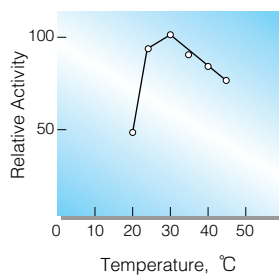
**Fig.3. pH-Activity**  
[ 25°C in 50mM buffer solution: pH3.0-5.5, acetate;  
pH5.5-8.5, phosphate; pH8.5-9.0, Tris-HCl  
pH10.0, sodium carbonate ]



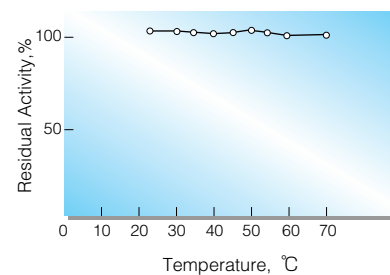
**Fig.5. pH-stability**  
[ 25°C, 20hr-treatment with 50mM buffer  
solution: (see Fig. 3) ]



**Fig.2. Stability (Liquid form at 5°C)**  
[ buffer composition : 10mM K-  
phosphate buffer, pH7.0 ]



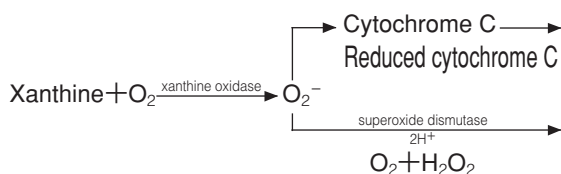
**Fig.4. Temperature activity**  
[ in 50mM phosphate buffer, pH7.0 ]



**Fig.6. Thermal stability**  
[ 30min-treatment with 50mM phosphate  
buffer, pH7.0 ]

## 活性測定法 (Japanese)

### 1.原理



還元型Cytochrome Cの生成量を550nmにおける吸光度の変化で測定する。

### 2.定義

下記条件下でCytochrome Cの還元を50%阻害する酵素量を1単位(U)とする

### 3.試薬

- 75mM K-リン酸緩衝液, pH7.8
  - 1.5mM EDTA水溶液 [55.8mgのEthylenediaminetetraacetate- $\text{Na}_2(2\text{H}_2\text{O})$ を蒸留水100mlに溶解する]
  - 0.75mM Xanthine溶液 [0.004N NaOH溶液に溶解する] (用時調整)
  - Xanthine oxidase溶液 [硫安懸濁液(約4U/ml)を蒸留水で0.04U/mlに希釈する] (用時調製)
  - 0.15mM Cytochrome C水溶液(馬心臓由来 Cytochrome Cを蒸留水に溶解する)(用時調整)
- 酵素溶液：酵素標品を予め氷冷した10mM K-リン酸緩衝液, pH7.8で溶解し,同緩衝液で0.5~2.0U/mlに希釈して氷冷保存する。

### 4.手順

- 下記反応混液をキュベット(d=1.0cm)に調製し,25°Cで約5分間予備加温する。
 

2.0ml	K-リン酸緩衝液	(A)
0.2ml	EDTA水溶液	(B)
0.2ml	Xanthine溶液	(C)
0.2ml	Cytochrome C水溶液	(E)
0.2ml	酵素溶液	(F)
- Xanthine oxidase溶液(D)0.20mlを添加し,ゆるやかに混和後,水を対照に25°Cに制御された分光光度計で550nmの吸光度変化を2~3分間記録し,その初期直線部分から1分間当りの吸光度変化を求める( $\Delta$  ODtest)。
- 盲検は反応混液①に酵素溶液の代わりに酵素希釈液(10mM K-リン酸緩衝液,pH7.8)を加え,上記同様に操作を行って,1分間当りの吸光度変化を求める( $\Delta$  ODblank)。

### 5.計算式

$$\begin{aligned}
 \text{U/ml} &= \left( \frac{\Delta \text{OD blank}}{\Delta \text{OD test}} - 1 \right) \times \frac{1}{0.2(\text{ml})} \times \text{希釈倍率} \\
 \text{U/mg} &= \text{U/ml} \times 1 / \text{C} \\
 \text{C} &: \text{溶解時の酵素濃度(c mg/ml)}
 \end{aligned}$$