●TOYOBO ENZYMES●

(Diagnostic Reagent Grade)

SUPEROXIDE DISMUTASE

from Bovine erythrocyte

Superoxide:superoxide oxidoreductase (EC 1.15.1.1)

$$2O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$$

PREPARATION and SPECIFICATION

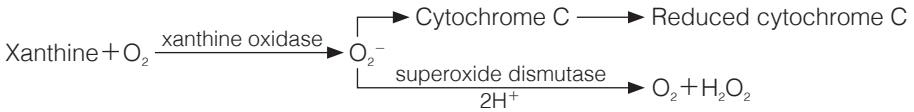
Appearance	: Bluish green amorphous powder, lyophilized
Activity	: Grade Ⅲ 3,000U/mg-solid or more
Contaminant	: Catalase ≤1.0×10^{-2}%

PROPERTIES

Stability	: Stable at −20℃ for at least one year (Fig.1) (A decrease in activity of ca.10% may occur within 6 months)
Molecular weight	: 32,000 [1]
Isoelectric point	: 4.95 [2]
Structure	: 2 subunits per enzyme molecule (Each one mole of copper and zinc is bound to each subunit)
Inhibitors	: Cyanide [4] ,diethyldithiocarbamate [5]
Optimum pH	: 9.0 (Fig.4)
Optimum temperature	: 30℃ (Fig.5)
pH Stability	: pH 7.0−8.5 (25℃, 20hr) (Fig.6)
Thermal stability	: below 70℃ (pH 7.0, 30min) (Fig.7)

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_2
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_2O] (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℃ 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	51	mM
Xanthine	50	μM
Cytochrome C	10	μM
EDTA	0.10	mM
Xanthine oxidase	2.6	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℃, 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 :

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

Weight activity (U/mg) = (U/ml)×1/C

Vs : 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)

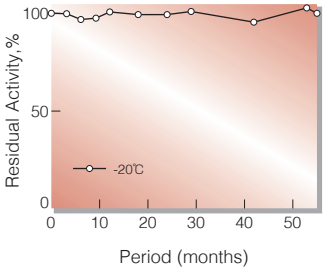


Fig.1. Stability (Powder form)
〔kept under dry conditions〕

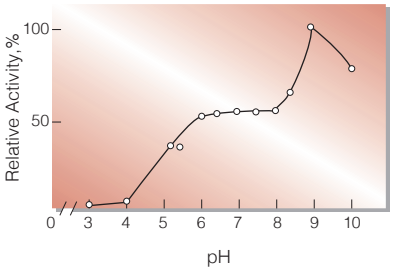


Fig.4. pH-Activity
〔25℃ in 50mM buffer solution: pH3.0-5.5, acetate; pH5.5-8.5, phosphate; pH8.5-9.0, Tris-HCl pH10.0,sodium carbonate〕

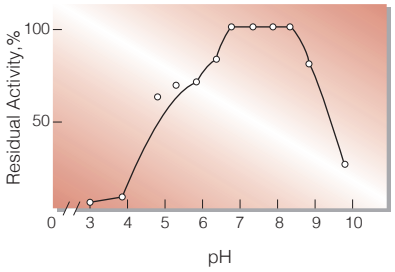


Fig.6. pH-stability
〔25℃, 20hr-treatment with 50mM buffer solution: (see Fig. 3)〕

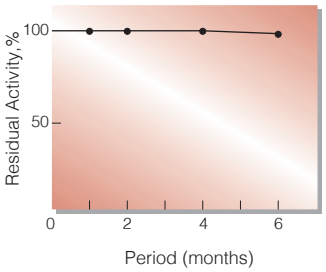


Fig.2. Stability (Powder form at 5℃)
〔kept under dry conditions〕

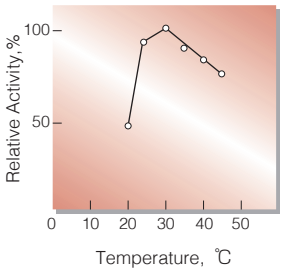


Fig.5. Temperature activity
〔in 50mM phosphate buffer, pH7.0〕

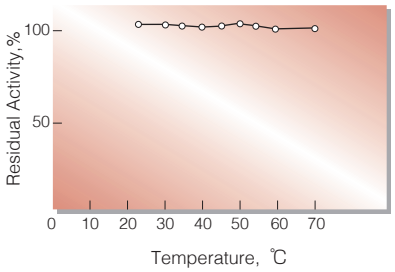


Fig.7. Thermal stability
〔30min-treatment with 50mM phosphate buffer, pH7.0〕

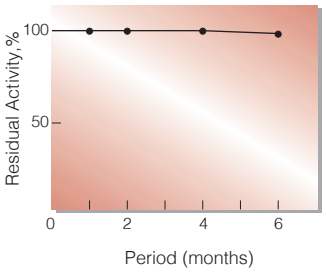
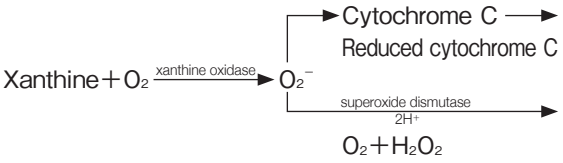


Fig.3. Stability (Liquid form at 5℃)
〔buffer composition : 10mM K-phosphate buffer, pH7.0〕

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

A. 75mM K-リン酸緩衝液, pH7.8
B. 1.5mM EDTA水溶液〔55.8㎎のEthylenediaminetetraacetate-$Na_2(2H_2O)$を蒸留水100㎖に溶解する〕
C. 0.75mM Xanthine溶液〔0.004N NaOH溶液に溶解する〕(用時調整)
D. Xanthine oxidase溶液〔硫安懸濁液(約4U/㎖)を蒸留水で0.04U/㎖に希釈する〕(用時調製)
E. 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/㎖に希釈して氷冷保存する。

4.手順

①下記反応混液をキュベット(d＝1.0cm)に調製し,25℃で約5分間予備加温する。

2.0㎖	K-リン酸緩衝液	(A)
0.2㎖	EDTA水溶液	(B)
0.2㎖	Xanthine溶液	(C)
0.2㎖	Cytochrome C水溶液	(E)
0.2㎖	酵素溶液	(F)

②Xanthine oxidase溶液(D)0.20㎖を添加し,ゆるやかに混和後,水を対照に25℃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(ΔODtest)。

③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(ΔODblank)。

5.計算式

$$U/㎖ = \left(\frac{\Delta OD\ blank}{\Delta OD\ test} - 1\right) \times \frac{1}{0.2(㎖)} \times 希釈倍率$$

U/mg ＝U/㎖×1/C

C ： 溶解時の酵素濃度(c ㎎/㎖)