

● TOYOBO ENZYMES ●
(Diagnostic Reagent Grade)

CATALASE

from Microorganism

Hydrogen-peroxide: hydrogen-peroxide oxidoreductase (EC 1. 11. 1. 6)



PREPARATION and SPECIFICATION

Appearance : Olive green solution
Activity : Grade V 150,000 U/ml or more

PROPERTIES

Stability	: Stable at 4°C	(Fig.1)
Molecular weight	: approx. 53,000 (by mass spectrometry)	
Inhibitors	: NaN_3	
Optimum pH	: 7.2–9.0	(Fig.2)
Optimum temperature	: 35–40°C	(Fig.3)
pH Stability	: pH 6.2–8.9 (25°C, 16hr)	(Fig.4)
Thermal stability	: below 35°C (pH 7.0, 30min)	(Fig.5)
Effect of various chemicals	: (Table 1)	

APPLICATIONS

This enzyme is useful for eliminating the interference of hydrogen-peroxide in clinical analysis.

ASSAY

Principle:



The disappearance of hydrogen peroxide is measured by the Titanium color method ¹⁾.

Unit definition:

One unit causes the hydrolysis of one micromole of hydrogen peroxide per minute under the conditions described below.

Method:

Reagents

- A. 10mM Phosphate buffer, pH 7.0 (at 25°C)
- B. H₂O₂ solution : 16mM [0.182ml of 30% (W/V)H₂O₂/100ml of buffer A] (Should be prepared fresh and store on ice.)
- C. Titanium reagent : (Nacalai tesque)
- D. Enzyme diluent : Buffer A

Procedure

1. Prepare 0.25ml of the substrate solution (B) into a test tube and equilibrate at 25°C for about 5 minutes
2. Add 0.25ml of the enzyme solution* and mix.
3. After exactly 5 minutes at 25°C, add 2.5ml of Titanium reagent (C) to stop the reaction and measure the optical density at 410nm against water (OD test)
At the same time, prepare the blank by first mixing the substrate solution with 2.5ml of Titanium reagent after 5 min-incubation at 25°C, followed by the addition of enzyme solution (OD blank)

* Dilute the enzyme preparation to 0.35–1.35U/ml with ice-cold enzyme diluent (D).

Calculation

Activity can be calculated by using the following formula :

$$\text{Volume activity (U/ml)} = \frac{\Delta \text{OD} (\text{OD blank} - \text{OD test}) \times V_t \times d_f}{F \times t \times 1.0 \times V_s} = \Delta \text{OD} \times 2.4 \times 1/F \times d_f$$

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

V_t : Total volume (3.0ml)

V_s : Sample volume (0.25ml)

F : Extinction coefficient of Titanium color product developed by the presence of 1.0mM hydrogen peroxide (F should be determined in each lot of Titanium reagent by using a known concentration of hydrogen peroxide. F is usually around 0.7.)

t : Reaction time (5 minutes)

1.0 : Light path length (cm)

d_f : Dilution factor

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

REFERENCES

- 1) F.Patti and P.B.-Maury; *Bull.Soc.Chem.Biol.*,35, 1177 (1953)

Table 1. Effect of Various Chemicals on Catalase

[The enzyme dissolved in 10mM K-phosphate buffer, pH 7.0 (200U/ml) was incubated with each chemical at 25°C for 1hr.]

Chemical	Concn.(mM)	Residual activity(%)	Chemical	Concn.(mM)	Residual activity(%)
None	—	100	NaF	2.0	89.3
Metal salt	2.0		NaN ₃	2.0	3.8
AgNO ₃		76.6	EDTA	5.0	96.6
BaCl ₂		99.6	IAA	2.0	99.4
CaCl ₂		62.4	Borate	20	98.8
CoCl ₂		101.2	SDS	0.05%	96.1
CuSO ₄		99.7	Triton X-100	0.10%	95.7
FeSO ₄		98.2	Brij 35	0.10%	97.8
MgSO ₄		99.2	Span 20	0.10%	94.5
MnCl ₂		28.7	Na-cholate	0.10%	95.3
NiCl ₂		53.6			
ZnCl ₂		96.1			

EDTA, ethylenediaminetetraacetate; IAA, iodoacetamide; SDS, sodium dodecyl sulfate; DAC, Dimethylbenzylalkylammonium chloride

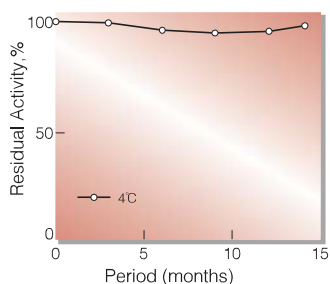


Fig.1. Stability (Liquid form)

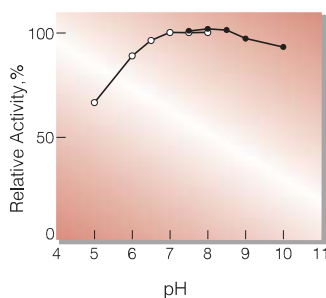


Fig.2. pH-Activity

[in 10mM buffer solution: pH 5-8,]
[K-phosphate: pH 7.5-10, Tris-HCl]

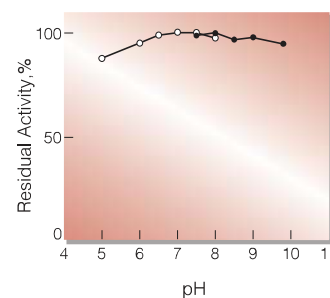


Fig.4. pH-Stability

[in 10mM buffer solution: pH 5-8,]
[K-phosphate: pH 7.5-10, Tris-HCl.]
[Enzyme concentration: 1U/ml]

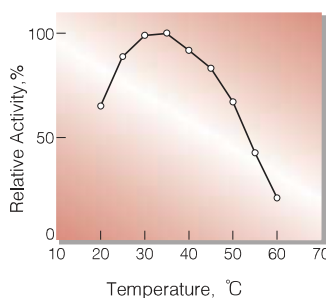


Fig.3. Temperature activity

[in 10mM K-phosphate buffer, pH 7.0]

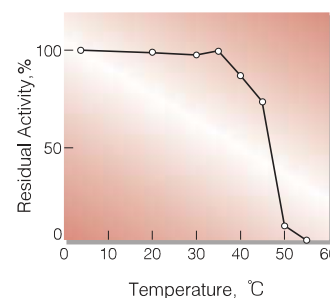


Fig.5. Thermal stability

[30min-treatment with 10mM K-phosphate buffer, pH 7.0.]
[Enzyme concentration: 200U/ml]

活性測定法 (Japanese)

1.原理



過酸化水素の減少量をチタン呈色法で測定する。

2.定義

下記条件下で1分間に1マイクロモルの過酸化水素を分解する酵素量を1単位(U)とする。

3.試薬

- A. 10mM リン酸緩衝液, pH 7.0
- B. H₂O₂溶液:16mM [0.182mlの30%(W/V)H₂O₂を100mlのbuffer Aに溶解する] (用時調製し, 使用時は氷冷保存する)
- C. チタン試薬:(ナカライテスク製)
- D. 酵素溶液:酵素備品を予め氷冷した緩衝液Aで0.35~1.35U/mlに希釈する。

4.手順

- ①試験管に0.25mlの基質溶液(B)を採り,25°Cで約5分間予備加温する。
- ②酵素溶液0.25mlを添加し,緩やかに混和する。
- ③25°Cで正確に5分間反応させた後,チタン試薬(C)2.5mlを加えて反応を停止させ,水を対照にして410nmの吸光度を測定する(OD test)。
- ④盲検は5分間の反応の後,最初に基質溶液(B)0.25mlをチタン試薬(C)2.5mlに加えて混和し,次いで酵素溶液を添加する(OD blank)。

5.計算式

$$\text{U/ml} = \frac{\Delta \text{OD} (\text{OD blank} - \text{OD test}) \times 3.0(\text{ml}) \times \text{希釈倍率}}{F \times 5(\text{分}) \times 1.0 \times 0.25(\text{ml})}$$

$$= \Delta \text{OD}/\text{min} \times 2.4 \times 1/F \times \text{df}$$

$$\text{U/mg} = \text{U/ml} \times 1/C$$

F : 0.1mM過酸化水素によるチタン呈色生成物の吸光係数 (Fは濃度の分かっている過酸化水素を用いて各ロット毎に決定する。通常は0.7前後である)

1.0 : 光路長(cm)

C : 溶解時の酵素濃度(c mg/ml)