# ●TOYOBO ENZYMES●
## (Diagnostic Reagent Grade)

# CATALASE
## *from Microorganism*

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

$$2H_2O_2 \rightleftharpoons O_2 + H_2O$$

### *PREPARATION and SPECIFICATION*

| | |
|---|---|
| Appearance | : Orive green solution |
| Activity | : Grade V 150,000 U/ml or more |

### *PROPERTIES*

| | | |
|---|---|---|
| Stability | : Stable at 4℃ | (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℃ | (Fig.3) |
| pH Stability | : pH 6.2－8.9 (25℃, 16hr) | (Fig.4) |
| Thermal stability | : below 35℃ (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:**

$2H_2O_2 \xrightarrow{\text{Catalase}} O_2 + 2H_2O$

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℃)

B. $H_2O_2$ solution : 16mM [0.182ml of 30% (W/V)$H_2O_2$/100ml of buffer A] (Should be prepare 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℃ for about 5 minutes
2. Add 0.25ml of the enzyme solution* and mix.
3. After exactly 5 minutes at 25℃, 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℃, follow 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 :

$$\text{Volume activity (U/ml)} = \frac{\Delta OD\ (OD\ blank - OD\ test) \times Vt \times df}{F \times t \times 1.0 \times Vs} = \Delta OD \times 3.43 \times 1/F \times df$$

Weight activity (U/mg) = (U/ml)×1/C

Vt : Total volume (3.0ml)

Vs : 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)

df : 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℃ for 1hr.]

| Chemical | Concn.(mM) | Residual activity(%) |
|---|---|---|
| None | — | 100 |
| Metal salt | 2.0 | |
| $AgNO_3$ | | 76.6 |
| $BaCl_2$ | | 99.6 |
| $CaCl_2$ | | 62.4 |
| $CoCl_2$ | | 101.2 |
| $CuSO_4$ | | 99.7 |
| $FeSO_4$ | | 98.2 |
| $MgSO_4$ | | 99.2 |
| $MnCl_2$ | | 28.7 |
| $NiCl_2$ | | 53.6 |
| $ZnCl_2$ | | 96.1 |

| Chemical | Concn.(mM) | Residual activity(%) |
|---|---|---|
| NaF | 2.0 | 89.3 |
| $NaN_3$ | 2.0 | 3.8 |
| EDTA | 5.0 | 96.6 |
| IAA | 2.0 | 99.4 |
| Borate | 20 | 98.8 |
| SDS | 0.05% | 96.1 |
| Triton X-100 | 0.10% | 95.7 |
| Brij 35 | 0.10% | 97.8 |
| Span 20 | 0.10% | 94.5 |
| Na-cholate | 0.10% | 95.3 |

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

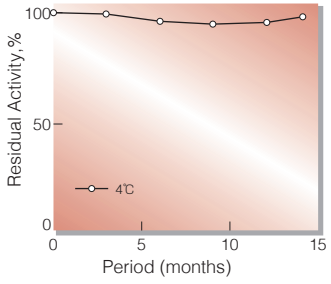


Fig.1. Stability (Liquid form)

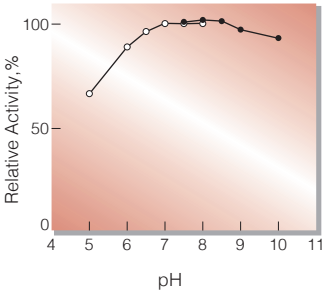


Fig.2. pH-Activity

〔in 10mM buffer solution: pH 5-8, K-phosphate; pH 7.5-10, Tris-HCl〕

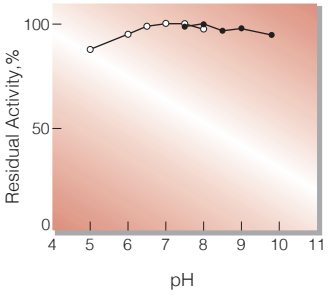


Fig.4. pH-Stability

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

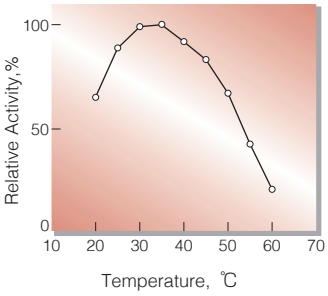


Fig.3. Temperature activity

〔in 10mM K-phosphate buffer, pH 7.0〕

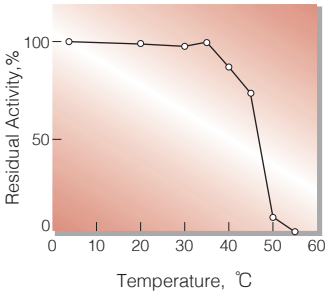


Fig.5. Thermal stability

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

## 活性測定法（Japanese）

### 1.原理

$2H_2O_2 \xrightarrow{Catalase} O_2 + 2H_2O$

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. $H_2O_2$溶液:16mM［0.182mℓの30%(W/V)$H_2O_2$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℃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℃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

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

$$= \Delta OD/min \times 3.43 \times 1/F \times df$$

$$U/mg = U/m\ell \times 1/C$$

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)