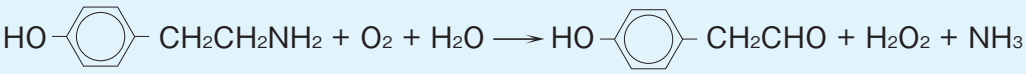
(Diagnostic Reagent Grade)

INQUIRY NEEDED
ASAHI KASEI ENZYMES T-25

TYRAMINE OXIDASE [TOD]

from *Arthrobacter* sp.
(Amine: oxygen oxidoreductase (deaminating) , EC 1.4.3.4)
(Amine oxidase)



Tyramine　　p-Hydroxybenzylaldehyde

Preparation and Specification

Appearance : White to light brownish amorphous powder, lyophilized
Specific activity : More than 3 U/mg solid

Properties

Substrate specificity	: See Table 1	
Molecular weight	: 71 kDa (SDS-PAGE) , 140 kDa (TSK gel G 3000 SWXL)	
Isoelectric point	: pH 4.23	
Michaelis constant	: Tyramine 2.7 × 10^{-5}M	
Optimum pH	: 7.0	Figure 1
pH stability	: 7.0–9.0 (37℃, 60 min)	Figure 2
Thermal stability	: Stable at 55℃ and below (pH 7.5, 5 min)	Figure 3

Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **leucine aminopeptidase** (LAP) using leucyltyramine as a substrate.

$$\text{Leucyltyramine} + H_2O \xrightarrow{\text{LAP}} \text{Leucine} + \text{Tyramine}$$

$$\text{Tyramine} + O_2 + H_2O \xrightarrow{\textbf{TOD}} \text{p-Hydroxybenzylaldehyde} + H_2O_2 + NH_3$$

$$2\,H_2O_2 + \text{4-AA} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + 4\,H_2O$$

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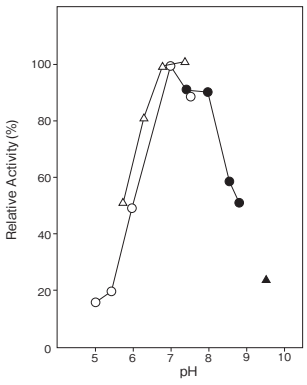
Table 1. Substrate specificity

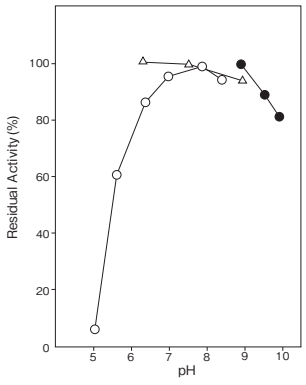
Substrate (1 mM)	Relative activity (%)	Km (mM)
Tyramine	100	0.027
Monoethanolamine	5	15
Trimethylamine	0	
Triethanolamine	0	
Glutamine	0	
Ethanediamine	0	
Dopamine	33	0.23
Histamine	68	0.48
Agmatine	9	0.26
Amylamine	132	0.20
Methylamine	0	
Phenylethylamine	141	0.003
Propylamine	0	
Arylamine	14	0.13
Benzylamine	0	
Hexylamine	115	0.10
1,4-Diaminobutane	0	
Cadaverine	0	

Fig.1 pH Optimum



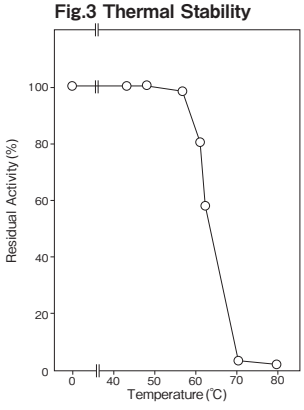
○ : 3,3-Dimethylglutarate-NaOH buffer
△ : Phosphate buffer
● : Tris-HCl buffer
▲ : Glycine-NaOH buffer

Fig.2 pH Stability



37℃, 60 min.
○ : 3,3-Dimethylglutarate-NaOH buffer
△ : Phosphate buffer
● : Tris-HCl buffer

Fig.3 Thermal Stability



pH 7.5, 5 min.
20 mM Phosphate buffer

Assay

■ Principle

The assay is based on the increase in absorbance at 480 nm as the formation of quinoneimine dye proceeds in the following reactions:

$$\text{Tyramine} + O_2 + H_2O \xrightarrow{\text{TOD}} p\text{-Hydroxybenzylaldehyde} + H_2O_2 + NH_3$$

$$2\,H_2O_2 + \text{4-AA} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + 4\,H_2O$$

■ Unit definition

One unit is defined as the amount of enzyme which oxidizes 1 μmole of tyramine to *p*-hydroxybenzylaldehyde per minute at 37℃ under the conditions specified in the assay procedure.

■ Reagents

1. Reaction mixture

0.1 M KH_2PO_4–NaOH buffer pH 7.5	0.20 ml
0.2% (W/V) Phenol solution	0.10 ml
15 mM 4-AA solution	0.10 ml
100 U/ml POD solution [1)]	0.05 ml
1 mM Tyramine solution	0.10 ml
Distilled water	0.45 ml

1): 100 U/ml POD solution
Dissolve 1,000 U (PPU) with 10 ml of distilled water.

2. Reaction stopper
Ethanol (not diluted)
3. Enzyme dilution buffer
20 mM KH_2PO_4 –NaOH buffer pH 7.5
4. Reagents
Tyramine: Tokyo Kasei Kogyo Co., Ltd. # A0302
4-AA: NACALAI TESQUE, INC. Special grade # 01907-52
POD: Sigma Chemical Co. Type II # P-8250

■ **Enzyme solution**

Accurately weigh about 20 mg of the sample and add enzyme dilution buffer to make a total of 20 ml. Dilute it with enzyme dilution buffer to adjust the concentration as required.

■ **Procedure**

1. Pipette accurately 1.0 ml of reaction mixture into a small test tube and preincubate at 37℃.
2. After 5 min, add exactly 50 μl of enzyme solution and mix to start the reaction at 37℃.
   ※ In the case of a test blank, add 50 μl of enzyme dilution buffer in place of enzyme solution.
3. At 10 min after starting the reaction, add 2.0 ml of the reaction stopper to stop the reaction.
4. Measure the absorbance at 480 nm.

Absorbance sample : As
blank : Ab
△A = (As − Ab) ≦ 0.30 Abs

■ **Calculation**

$$\text{Activity (U/mg of powder)} = \frac{\triangle A/10}{17.17 \times 1/2} \times \frac{3.05}{0.05} \times \frac{1}{X}$$

17.17 : millimolar extinction coefficient of quinoneimine dye at 480 nm (cm^2 / μmole)
1/2 : a multiplier derived from the fact that 2 mole of H_2O_2 produces 1 mole of quinoneimine dye
10 : reaction time (min)
3.05 : final volume (ml)
0.05 : volume of enzyme solution (ml)
X : concentration of the sample in enzyme solution (mg/ml)

Storage

Storage at −20℃ in the presence of a desiccant is recommended.

References

1. Kumagai, H., Matsui, H., Ogata, K. and Yamada, H., (1969) Biochim. Biophys. Acta, **171**, 1–8.
2. Yamada, H., Kumagai, H., Uwajima, T. and Ogata, K. (1967) Agric. Biol. Chem., **31**, 897–901.
3. Yamada, H., Kumagai, H. and Uwajima, T. (1971) Methods Enzymol., **17B**, 722–726.
4. Kumagai, H., Yamada, H., Suzuki, H., and Ogata, Y. (1971) J. Biochem. (Tokyo), **69**, 137–144.

TOD 活性測定法 (Japanese)

Ⅰ. 試薬液

1. 反応試薬混合液

0.1M KH_2PO_4–NaOH 緩衝液 pH7.5	0.20 ml
0.2% (W/V) フェノール溶液	0.10 ml
15mM 4–AA 溶液	0.10 ml
100U/ml POD 溶液 [1)]	0.05 ml
1mM チラミン溶液	0.10 ml
精製水	0.45 ml

1): 100U/ml POD 溶液
POD 1,000 単位 (PPU) を精製水 10ml で溶解する。

2. 反応停止液
エタノール原液を使用する。
3. 酵素溶解希釈用液
20mM KH_2PO_4–NaOH 緩衝液 pH7.5
4. 試薬
チラミン：東京化成製 #A0302
4–AA：ナカライテスク製 特級 #01907–52
POD：シグマ製 Type Ⅱ #P–8250

Ⅱ. 酵素試料液

検品約 20mg を精密に量り、酵素溶解希釈用液で全容 20ml とする。
その液を酵素溶解希釈用液で適宜希釈する。

Ⅲ. 測定操作法

1. 小試験管に反応試薬混合液 1.0ml を正確に分注し、37℃で予備加温する。
2. 5 分経過後、酵素試料液 50 μl を正確に加えて混和し、37℃で反応を開始する。
   ※盲検は酵素試料液の代わりに酵素溶解希釈用液 50 μl を加える。
3. 10 分経過後、反応停止液 2.0ml を加えて混和し、反応を停止する。
4. 480nm における吸光度を測定する。
   求められた吸光度を試料液は As、盲検液は Ab とする。

$$\Delta A = (As - Ab) \leqq 0.30 \text{ Abs}$$

Ⅳ. 計算

$$\text{活性 (U/mg)} = \frac{\Delta A/10}{17.17 \times 1/2} \times \frac{3.05}{0.05} \times \frac{1}{X}$$

17.17 : キノンイミン色素の 480nm におけるミリモル分子吸光係数 (cm^2/ μmole)
1/2 : H_2O_2 2 モルから キノンイミン色素 1 モルが生成することによる係数
10 : 反応時間 (min)
3.05 : 反応総液量 (ml)
0.05 : 反応に供した酵素試料液量 (ml)
X : 酵素試料液の検品濃度 (mg/ml)

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