(Diagnostic Reagent Grade) T−209

OXALOACETATE DECARBOXYLASE [OAC Ⅱ]

from *Pseudomonas* sp.
(Oxaloacetate carboxy-lyase, EC 4.1.1.3)
(Long-chain-fatty-acid-CoA ligase)

Oxaloacetate ⟶ Pyruvate + CO_2

Preparation and Specification

Appearance	: White to off white amorphous powder, lyophilized
Specific activity	: More than 2300 U/mg solid
Contaminants	:
AST (GOT)	Less than 0.005 % (U/U)
Catalase	Less than 0.3 % (U/U)

Properties

Molecular weight	: 31 kDa (SDS-PAGE), 120 kDa (Superdex 200)	
Isoelectric point	: pH 5.16	
Michaelis constant	: Oxaloacetate 3.3 × 10^{-3}M	
Optimum pH	: 7.5–8.5	Figure 1
pH stability	: 7.5–9.0 (50℃, 3 hr.)	Figure 2
Optimum temperature	: 40–50℃	Figure 3
Thermal stability	: Stable at 50℃ and below (pH 7.0, 10 min)	Figure 4
Storage stability	: At least one year at −20℃	
Activators	: Mg^{2+}, Mn^{2+}	
Inhibitors	: Sodium dodecylsulfate, Sodium laurylbenzen sulfonate	

Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **AST** when coupled with pyruvate oxidase (T-45).

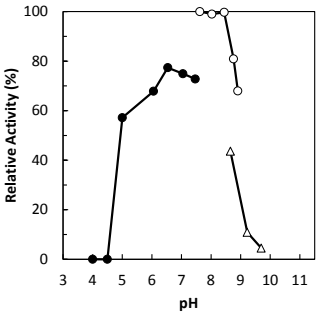
$$\text{Aspartic acid} + \alpha\text{-ketoglutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate} + \text{Glutamic acid}$$

$$\text{Oxaloacetate} \xrightarrow{\textbf{OAC}\,\textbf{Ⅱ}} \text{Pyruvate} + CO_2$$

$$\text{Pyruvate} + O_2 + H_2O \xrightarrow{\text{POPG}} \text{Acetylphosphate} + CO_2 + H_2O_2$$

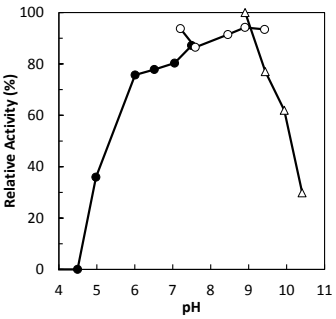
$$2\,H_2O_2 + 4\text{-AA} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + 4\,H_2O$$

Fig.1 pH Optimum



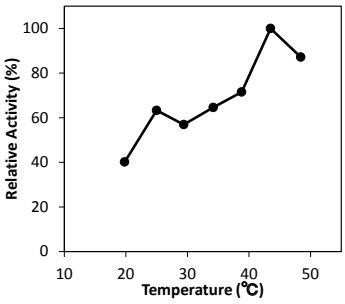
● : 3,3-Dimethylglutarate-NaOH buffer
○ : Tris-HCl buffer
△ : Glycine-NaOH buffer

Fig.2 pH Stability



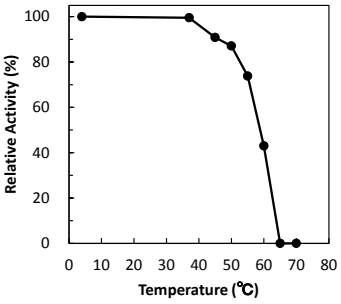
50℃, 3 hr.
● : 3,3-Dimethylglutarate-NaOH buffer
○ : Tris-HCl buffer
△ : Glycine-NaOH buffer

Fig.3 Optimum Temperature



pH 8.0
0.1 M TEA-NaOH buffer

Fig.4 Thermal Stability



pH 7.0, 10 min.
50 mM phosphate buffer

Assay

■ Principle

The assay is based on the decrease in absorbance at 340 nm as the consumption of NADH proceeds in the following reactions:

$$\text{Oxaloacetate} \xrightarrow{\text{OAC II}} \text{Pyruvate} + CO_2$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{Lactate} + NAD^+$$

LDH: Lactate dehydrogenase
NADH: Nicotineamide adenine dinucleotide

■ Unit definition

One unit is defined as the amount of enzyme which produces 1 μmole of pyruvate per minute at 25℃ under the conditions specified in the assay procedure.

■ Reagents

1. Reaction mixture

0.1 M TEA-NaOH buffer pH 8.0	0.90 ml
10 mM $MnCl_2$ solution	0.10 ml
6.5 mM NADH solution	0.10 ml
Distilled water	0.90 ml

2. 550 U/ml LDH solution
   Mix 0.1 ml of LDH solution (10 mg/2 ml, 2,750 U/ml) with 0.4 ml of 10 mM Tris-HCl buffer pH 7.0
3. Substrate solution (50 mM oxaloacetate solution)
   Dissolve 33 mg of oxaloacetate with 5 ml of 0.2 M Tris-HCl buffer pH 9.0.
4. Enzyme dilution buffer
   10 mM Tris-HCl buffer pH 8.0
5. Reagents
   TEA (Triethanolamine, HCl salt) : Merck Co.
   $MnCl_2 \cdot 4H_2O$:
   FUJIFILM Wako Pure Chemical Corporation
   Special grade #133-00725
   NADH (2Na・$3H_2O$, Reduced form):
   Kyowa Hakko Co., Ltd.
   LDH: Roche Diagnostics GmbH #10 127 876 001
   Oxaloacetate:
   FUJIFILM Wako Pure Chemical Corporation #150-00411

■ 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 to within 0.5–1.4 U/ml.

■ Procedure

1. Pipette accurately 2.0 ml of reaction mixture, 20 μl of LDH solution and 50 μl of enzyme solution into a samll test tube and preincubate at 25℃.
   ※ In the case of a test blank, add 50 μl of enzyme dilution buffer in place of enzyme solution.
2. After 5 min, add 100 μl of substrate solution and mix to start the reaction at 25℃.
3. After starting the reaction, measure the rate of decrease per minute in absorbance at 340 nm. The rate must be measured within the linear portion of the absorbance curve.

Absorbance sample : As/min
blank : Ab/min

$\triangle A/\text{min} = (As/\text{min} - Ab/\text{min}) \leqq 0.15$ Abs/min

■ Calculation

$$\text{Activity (U/mg of powder)} = \frac{\triangle A/\text{min}}{6.22} \times \frac{2.17}{0.05} \times \frac{1}{X}$$

6.22 : millimolar extinction coefficient of NADH at 340 nm (cm^2/ μmole)
2.17 : 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. Enzyme activity will be retained for at least one year under this condition.

References

1. Horton, A. A. and Kornberg, H. L. (1964) Biochem. Biophys. Acta, **89**, 381–383.
2. Schmitt, A., Bottke, I. and Siebert, G. (1966) Hoppe–Seyler's Z. Physiol. Chem., **347**, 18–34.

OAC Ⅱ活性測定法（Japanese）

Ⅰ．試薬液

1. 反応試薬混合液

0.1M TEA–NaOH 緩衝液　pH8.0	0.90 ml
10mM 塩化マンガン溶液	0.10 ml
6.5mM NADH 溶液	0.10 ml
精製水	0.90 ml

2. 550U/ml LDH 溶液
   ロシュ製 LDH 溶液（10mg/2ml 2,750U/ml）の 0.1ml と 10mM トリス –HCl 緩衝液 pH7.0 を 0.4ml 混合する。
3. 基質溶液（50mM オキサロ酢酸溶液）
   オキサロ酢酸 33mg を 0.2M トリス–HCl 緩衝液 pH9.0 5ml で溶解する。
4. 酵素溶解希釈用液
   10mM トリス–HCl 緩衝液 pH8.0
5. 試薬
   TEA（トリエタノールアミン・塩酸塩）：メルク製
   塩化マンガン（$MnCl_2 \cdot 4H_2O$）：
   　　富士フイルム和光純薬製　特級　#133–00725
   NADH（ニコチンアミドアデニンジヌクレオチド・2Na・$3H_2O$・還元型）：協和発酵製
   LDH（乳酸脱水素酵素）：ロシュ製　#10 127 876 001
   オキサロ酢酸：
   　　富士フイルム和光純薬製　#150-00411

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溶解して全容 20ml とする。その液を酵素溶解希釈用液で 0.5～1.4U/ml 濃度となるように適宜希釈する。

Ⅲ．測定操作法

1. 小試験管に反応試薬混合液 2.0ml と LDH 溶液 20 μl 及び酵素試料液 50 μl を正確に分注して 25℃で予備加温する。
   ※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。
2. 5 分経過後、基質溶液 100 μl を加えて混和し、25℃で反応を開始する。
3. 反応開始後、340nm における吸光度を測定して直線的に反応している 1 分間当たりの吸光度変化を求める。
   求められた吸光度変化を試料液は As/min、盲検液は Ab/min とする。
   $\Delta A/min = (As/min - Ab/min) \leqq 0.15$ Abs/min

Ⅳ．計算

$$活性\ (U/mg) = \frac{\Delta A/min}{6.22} \times \frac{2.17}{0.05} \times \frac{1}{X}$$

6.22 : NADH の 340nm におけるミリモル分子吸光係数（$cm^2/\ \mu mole$）
2.17 : 反応総液量（ml）
0.05 : 反応に供した酵素試料液量（ml）
X　: 酵素試料液中の検品濃度（mg/ml）

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