(Diagnostic Reagent Grade)

T-249

Hippuricase [HIP Ⅱ]

from Microorganism
(Hippurate hydrolase, EC 3.5.1.32)

p-Hydroxybenzoylglycine ⟶ *p*-Hydroxybenzoic acid + Glycine

Preparation and Specification

Appearance : White to pale brownish lyophilizate
Specific activity : More than 80 U/mg solid

Properties

Michaelis constant : 2.7×10^{-3} M (*p*-Hydroxybenzoyl glycine)
Optimum pH : See Figure 1
pH stability : See Figure 2
Optimum temperature : See Figure 3
Thermal stability : See Figure 4

Applications for Diagnostic Test

This enzyme is useful for enzymatic quantification of angiotensin-converting enzyme (ACE). HC-ANC Ⅱ (T-256) is also available as a calibrator for ACE test.

Fig.1 Optimum pH

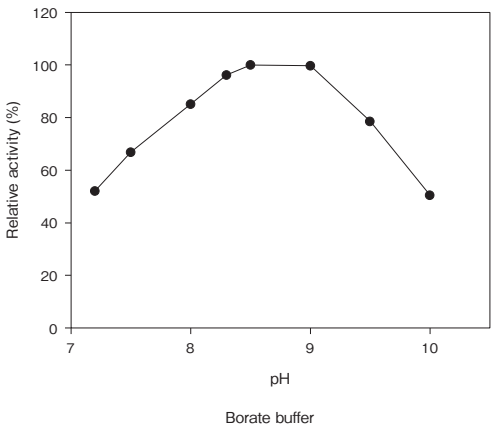

Fig.2 pH Stability

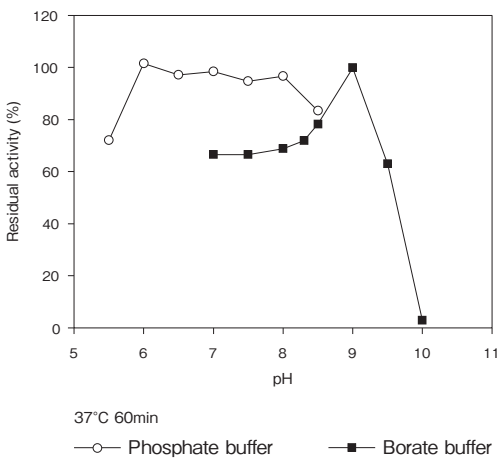

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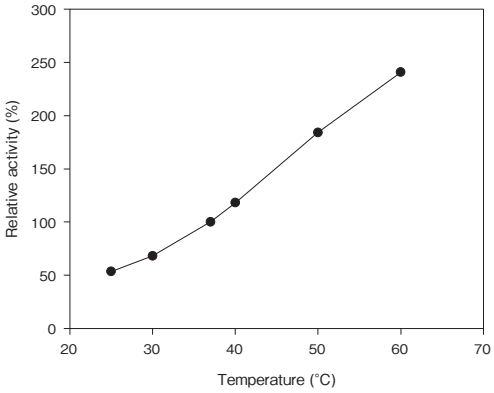
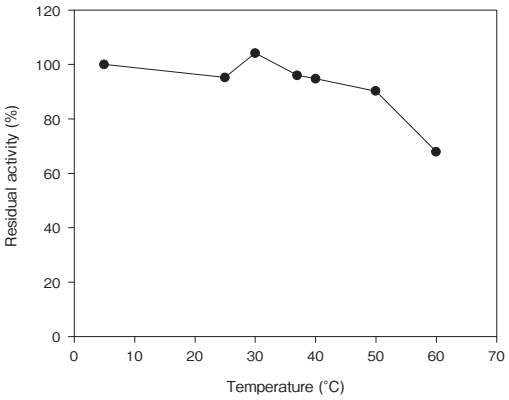
Fig.3 Optimum temperature



Borate buffer pH 8.3

Fig.4 Thermal stability



Phosphate buffer pH 7.0, 30min

Assay

■ Principle

The assay is based on the enzymatic hydrolysis of *p*-hydroxybenzoylglycine to produce *p*-hydroxybenzoic acid, followed by oxidative coupling to form a quinoneimine dye. The produced quinoneimine dye is measured spectrophotometrically at 505 nm

HIP Ⅱ

p-Hydroxybenzoylglycine → *p*-Hydroxybenzoic acid + Glycine

$NaIO_4$

p-Hydroxybenzoic acid + 4-Aminoantipyrine → Quinoneimine dye

■ Unit Definition

One unit is defined as the amount of enzyme that produces 1 μ mol of *p*-hydroxybenzoic acid per minute at 37℃ under the conditions specified in the assay procedure.

■ Reagents

1. Reaction mixture
   Dissolve 742 mg of boric acid and 4.09 g of NaCl in 80 mL of purified water. Adjust the pH to 8.3 (25° C) with 4N NaOH, then make up the volume to 100 mL with purified water (borate buffer). Dissolve 195 mg of *p*-hydroxybenzoyl glycine in the borate buffer, then adjust the pH to 8.3 (25° C) with 4 N NaOH. Add 25 mg of 4-Aminoantipyrine (4-AA) to this solution and dissolve, then make up the volume to 50 mL with the borate buffer.
2. Stopping & coloring agent
   Dissolve 112 mg of EDTA and 0.2 g of Triton X-100 in purified water and make up the total volume to 100 mL. Immediately before use, add 139 mg of $NaIO_4$ to 100 mL of this solution and dissolve.
3. Enzyme dilution buffer
   0.12 M borate-NaOH buffer (pH 8.3) containing 0.7M NaCl and 0.1% BSA
4. Reagents
   Boric acid: FUJIFILM Wako Pure Chemical #021-02195
   4-Hydroxy-hippuric acid: Bachem AG #4005059.0001
   4-AA: NACALAI TESQUE #01907-52
   Triton X-100: Dow Chemical
   NaIO4: FUJIFILM Wako Pure Chemical #197-02402
   EDTA: KISHIDA CHEMICAL #060-29133
   NaCl: FUJIFILM Wako Pure Chemical #191-01665
   BSA: Milipore #81-053

■ Enzyme solution

Accurately weigh about 10 mg of the sample and add enzyme dilution buffer to make a total of 10 ml. Dilute it with enzyme dilution buffer to adjust the concentration as required.

■ Procedure

1. Pipette 0.50 mL of reaction mixture into a test tube and preincubate at 37℃.
2. After 5 min, add 50 μ L of enzyme solution to start the reaction at 37℃.
   * In the case of a test blank, add 0.50 μ L of enzyme dilution buffer in place of enzyme solution.
3. Incubate at 37oC for 10 minutes. Add 1.50 mL stop/color reagent and mix at 37℃.
4. Measure absorbance at 505 nm.
   Absorbance sample : As
   Blank : Ab
   △ A = As − Ab ≤ 0.400

■ **Calculation**

$$\text{Activity (U/mg of powder)} = \frac{\Delta A/10}{12} \times \frac{2.05}{0.05} \times \frac{1}{X} \times 1.5$$

12 : molar extinction coefficient of quinoneimine at 505 nm (cm^2/μ mol)
10 : reaction time (min)
2.05 : final volume (mL)
0.05 : volume of enzyme solution (mL)
X : concentration of the sample in enzyme solution (mg/mL)
1.5 : correction factor

HIP Ⅱ活性測定法（Japanese）

Ⅰ．試薬液

1. 反応試薬混合液
   ホウ酸 742 mg および NaCl 4.09 g を精製水 80 mL に溶解する。4N NaOH を用いて pH を 8.3（25℃）に調整した後、精製水で全量を 100 mL とする（ホウ酸緩衝液）。このホウ酸緩衝液に *p*-ヒドロキシベンゾイルグリシン 195 mg を溶解し、4N NaOH で pH を 8.3（25℃）に調整する。続いて 4-AA 25 mg を加えて溶解し、ホウ酸緩衝液で全量 50 mL とする。
2. 反応停止･呈色試薬
   EDTA 112 mg および Triton X-100 0.2 g を精製水に溶解し、全量を 100 mL とする。使用直前に、この溶液 100 mL に対して $NaIO_4$ 139 mg を加え、溶解する。
3. 酵素溶解希釈用液
   0.1% BSA を含むホウ酸緩衝液
4. 試薬
   ホウ酸：富士フイルム和光純薬製 #021-02195
   4-Hydroxy-hippuric acid: Bachem AG #4005059.0001
   4-AA: ナカライテスク #01907-52
   Triton X-100: Dow Chemical
   NaIO4: 富士フイルム和光純薬製 #197-02402
   EDTA: キシダ化学 #060-29133
   NaCl: 富士フイルム和光純薬製 #191-01665
   BSA: Milipore #81-053

Ⅱ．酵素試料液

検品約 10mg を精密に量り、酵素溶解希釈用液に溶解して全容 10ml とする。
その液を酵素溶解希釈用液で適切な濃度へと適宜希釈する。

Ⅲ．測定操作法

1. 試験管に反応試薬混合液 0.50 mL を分注し、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 0.50 mL を加える。
3. 37℃で 10 分間反応させる。その後、停止･呈色試薬 1.50 mL を加え、37℃で混合する。
4. 505 nm 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。
   $\Delta A = As - Ab \leqq 0.400$

Ⅳ．計算

$$\text{活性 (U/mg)} = \frac{\Delta A/10}{12} \times \frac{2.05}{0.05} \times \frac{1}{X} \times 1.5$$

12 : 505 nm におけるキノンイミンのモル吸光係数（cm^2/μ mol）
10 : 反応時間（min）
2.05 : 反応総液量（mL）
0.05 : 反応に供した酵素試料液量（mL）
X : 酵素試料液中の検品濃度（mg/mL）
1.5 : 補正係数