Histamine Dehydrogenase (HDH-E)

from recombinant *E. coli*

Histamine : (acceptor) oxidoreductase (deaminating), EC 1.4.99

$$\text{Histamine} + \text{Acceptor} + H_2O \longrightarrow \text{4-Imidazolylacetoaldehyde} + \text{Reduced acceptor} + NH_3$$

SPECIFICATION

Appearance	brownish lyophilizate
Activity	≧1 U/mg lyophilizate
Stabilizer	trehalose
Storage	at −20°C

PROPERTIES

Molecular weight	*ca.* 150 kDa (gel filtration)	
Structure	2 subunits of *ca.* 71 kDa (SDS-PAGE)	
Michaelis constant	1.0×10^{-5} M (histamine)	
pH Optimum	9.0–9.5	(Fig. 1)
pH Stability	5.5–10.5	(Fig. 2)
Optimum temperature	70°C	(Fig. 3)
Thermal stability	below 65°C	(Fig. 4)
Stability (liquid form)	stable at 37°C for at least four weeks	(Fig. 5)
Stability (powder form)	stable at 50°C for at least two weeks	(Fig. 6)
Specificity	histamine (100), agmatine (7) putrescine (0), cadaverine (0)	

ASSAY PROCEDURE

Principle

$$\text{Histamine} + \text{1-Methoxy PMS} + H_2O \xrightarrow{\text{histamine dehydrogenase}} \text{4-Imidazolylacetoaldehyde} + \text{1-Methoxy PMSH}_2 + NH_3$$

$$\text{1-Methoxy PMSH}_2 + \text{WST-8 (ox)} \longrightarrow \text{1-Methoxy PMS} + \text{WST-8 (red)}$$

The appearance of WST-8 (red) is measured spectrophotometrically at 460 nm.

Definition of unit

One unit (U) is defined as the amount of enzyme which produces 1 μmol of 4-imidazolylacetoaldehyde per min at 37°C and pH 9.0 under the conditions described below.

Reagents

A. Glycine-NaOH buffer, 0.1 M; pH 9.0: dissolve 751 mg of glycine in 80 ml of distilled water, adjust to pH 9.0 with 1 N NaOH and dilute with distilled water to 100 ml.
B. 1-Methoxy PMS solution, 0.3 mM: 10 mg of 1-Methoxy PMS/100 ml of distilled water. (protect from light)
C. WST-8 solution, 1 mM: 60 mg of WST-8/100 ml of distilled water.
D. Histamine solution: dissolve 169 mg of histamine dihydrochloride in 80 ml of distilled water, adjust to pH 7.0 with 1 N NaOH and dilute with distilled water to 100 ml.
E. Enzyme dilution buffer: dissolve 375 mg of glycine in 80 ml of distilled water, adjust to pH 9.0 with 1 N NaOH and dilute with distilled water to 100 ml.

Sample: dissolve the lyophilized enzyme to a volume activity of 0.02–0.10 U/ml in ice-cold enzyme dilution buffer (Reagent E) immediately before measurement.

Procedure

1. Pipette the following reagents into a test tube.

2.4 ml Glycine-NaOH buffer	(Reagent A)
0.1 ml 1-Methoxy PMS solution	(Reagent B)
0.3 ml WST-8 solution	(Reagent C)
0.1 ml Histamine solution	(Reagent D)

2. Equilibrate at 37°C for about 5 min.
3. Add 0.1 ml of sample and mix.
4. Record the increase of absorbance at 460 nm in a spectrophotometer thermostated at 37°C, and calculate the ΔA per min using the linear portion of the curve (ΔA_S).
   The blank solution is prepared by adding enzyme dilution buffer (Reagent E) instead of sample (ΔA_0).

Calculation

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{(\Delta A_S - \Delta A_0) \times 3.0(\text{ml}) \times df}{36 \times 0.1(\text{ml})} = \Delta A \times 0.83 \times df$$

Weight activity (U/mg) = (U/ml) × 1/*C*

36 : Millimolar extinction coefficient of WST-8 (red) under the assay conditions ($cm^2/\mu mol$)
df : Dilution factor
C : Content of histamine dehydrogenase preparation in sample (mg/ml)

APPLICATIONS

The enzyme is useful for the determination of histamine in food analysis and in clinical analysis.

$$\text{Histamine} + \text{1-Methoxy PMS} + H_2O \xrightarrow{\text{histamine dehydrogenase}} \text{4-Imidazolylacetoaldehyde} + \text{1-Methoxy PMSH}_2 + NH_3$$

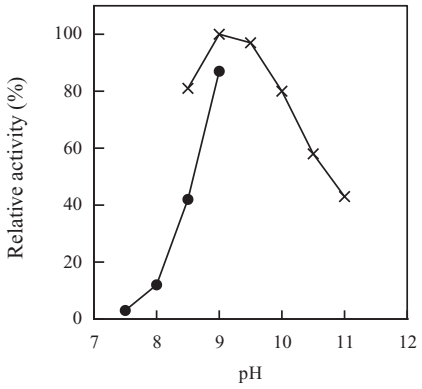
$$\text{1-Methoxy PMSH}_2 + \text{WST-8 (ox)} \longrightarrow \text{1-Methoxy PMS} + \text{WST-8 (red)}$$

REFERENCE

Ishiyama, M. *et al.*, *Talenta*, **44**, 1299–1305 (1997).

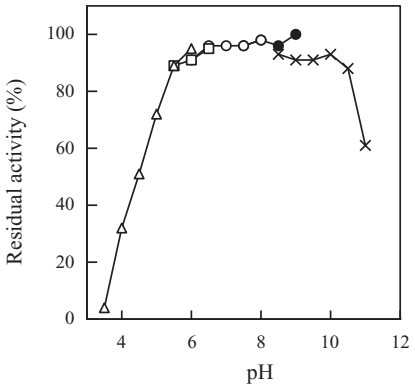
EXPERIMENTAL DATA

Fig. 1 pH Optimum



●: 0.1 M Tris-HCl buffer
×: 0.1 M Glycine-NaOH buffer

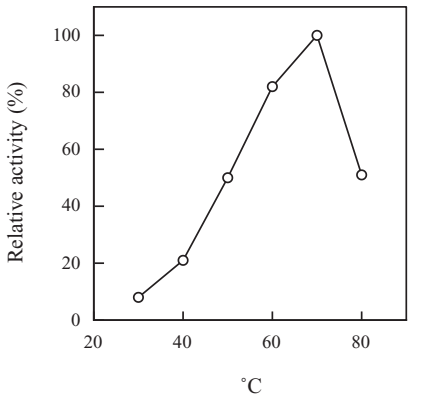
Fig. 2 pH Stability



Treatment: 37˚C, 16 h

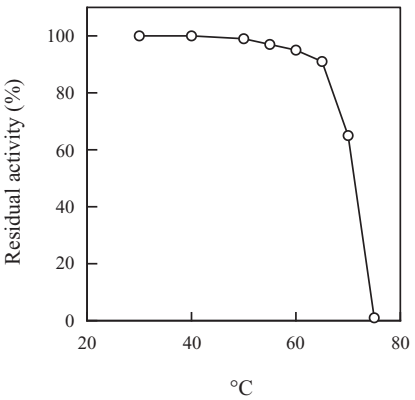
△: 0.1 M acetate buffer
□: 0.1 M MES-NaOH buffer
○: 0.1 M phosphate buffer
●: 0.1 M Tris-HCl buffer
×: 0.1 M Glycine-NaOH buffer

Fig. 3 Optimum temperature



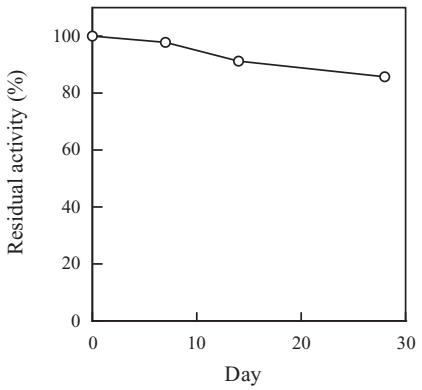
Buffer: 0.1 M Glycine-NaOH buffer, pH 9.0

Fig. 4 Thermal stability



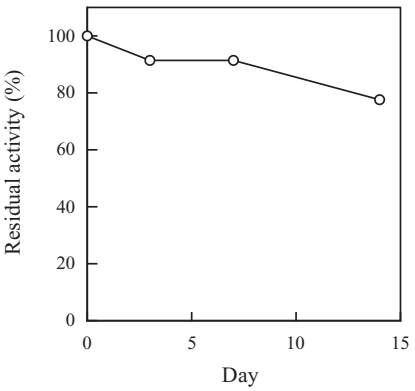
Treatment: 50 mM Glycine-NaOH buffer, pH 9.0

Fig. 5 Stability (liquid form) at 37°C



(Kept in 20 mM potassium phosphate buffer, pH8.0)

Fig. 6 Stability (powder form) at 50°C



(Kept under dry conditions)