Enzymatic Assay of HISTIDASE
(EC 4.3.1.3)

PRINCIPLE:

$_{L}$-Histidine $\xrightarrow{\text{Histidase}}$ Urocanic Acid + NH_3

CONDITIONS: T = 25°C, pH 9.0, A_{277nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 200 mM Tris HCl Buffer, pH 9.0 at 25°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 9.0 at 25°C with 1 M HCl.)

B. 10 mM Magnesium Chloride Solution, with 10 mM Tris HCl, pH 9.0 at 25°C ($MgCl_2$)
(Prepare 10 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250 and Reagent A. Adjust to pH 9.0 at 25° with 1 M HCl or 1 M NaOH, if necessary.)

C. 100 mM Reduced Glutathione Solution, pH 9.0 at 25°C (GSH)
(Prepare 10 ml in Reagent A using Glutathione, Reduced Form, Free Acid, Sigma Prod. No. G-4251. Adjust to pH 9.0 at 25°C with 100 mM NaOH.)

D. 100 mM Tris HCl Buffer, pH 9.0 at 25°C (Enzyme Diluent)
(Prepare 50 ml in deionized water using Reagent A. Adjust to pH 9.0 at 25°C with 1 M HCl or 1 M NaOH, if necessary.)

E. 200 mM $_{L}$-Histidine Substrate Solution, pH 9.0 at 25°C (Hist)
(Prepare 10 ml in Reagent A using $_{L}$-Histidine, Monohydrochloride, Monohydrate, Sigma Prod. No. H-8125. Adjust to pH 9.0 at 25°C with 100 mM NaOH or 100 mM HCl. **Prepare Fresh**.)

F. Histidase Enzyme Solution
(Immediately before use, prepare a solution containing 200 - 500 units/ml of Histidase in cold Reagent D.)

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PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

	Test	Blank
Reagent A (Buffer)	0.50	0.50
Reagent B ($MgCl_2$)	1.00	1.00
Reagent C (GSH)	0.10	0.10
Reagent F (Enzyme Solution)	0.05	------
Reagent D (Enzyme Diluent)	------	0.05
Deionized Water	0.95	0.95

Mix by inversion and incubate at 25°C for 30 minutes. This amount of time is required to activate the enzyme. Monitor the A_{277nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent E (Hist)	0.40	0.40

Immediately mix by inversion and record the increase in A_{277nm} for approximately 5 minutes. Obtain the ΔA_{277nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{277nm}/\text{min Test} - \Delta A_{277nm}/\text{min Blank})(3)(df)}{(0.0188)(0.05)}$$

3 = Volume (in milliliters) of assay
df = Dilution factor
0.0188 = µmolar extinction coefficient of Urocanic Acid at 277 nm
0.05 = Volume (in milliliters) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will deaminate 1.0 nanomole (10^{-9} mole) of L-histidine to urocanic acid per minute at pH 9.0 at 25°C.

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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 71 mM Tris, 3.3 mM magnesium chloride, 3.3 mM reduced glutathione, 27 mM L-histidine, and 10 - 25 units histidase.

REFERENCE:

Hassall, H. (1971) *Methods in Enzymology*, XVII B, 895-897.

NOTES:

1. This assay is based on the cited reference.

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.