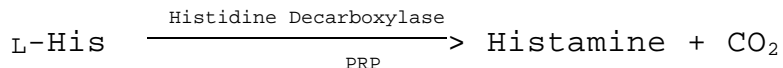


**Enzymatic Assay of L-HISTIDINE DECARBOXYLASE
(EC 4.1.1.22)**

PRINCIPLE:



Abbreviations:

L-His = L-Histidine

PRP = Pyridoxal 5-Phosphate

CONDITIONS: T = 37°C, pH 4.5

METHOD: Manometric Assay using Warburg Flasks

Reagents:

- A. 80 mM Sodium Acetate Buffer, pH 4.5 at 37°C
(Prepare 100 ml in deionized water using Sodium Acetate Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 4.5 at 37°C with 1 M HCl.)
- B. 100 mM L-Histidine Solution (L-His)
(Prepare 50 ml in Reagent A using L-Histidine, Free Base, Sigma Prod. No. H-8000. Adjust the pH to 4.5 with 1 M HCl, if necessary.)
- C. 61 mM Pyridoxal 5-Phosphate Solution (PRP)
(Prepare 25 ml in Reagent A using Pyridoxal 5-Phosphate, Sigma Prod. No. P-9255. Adjust the pH to 4.5 at 37°C with either 1 M HCl or 1 M NaOH.)
- D. Histidine Decarboxylase Enzyme Solution
(Immediately before use, prepare a solution containing 4 - 10 units/ml of L-Histidine Decarboxylase in cold deionized water.)

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PROCEDURE:

Pipette (in milliliters) the following reagents into Warburg flasks:

Main Chamber	Thermo- barometer <u>Flask</u>	Enzyme <u>Blank</u>	<u>Test</u> ¹	Substrate <u>Blank</u>
Reagent A (Buffer)	2.80	2.80	0.30	0.30
Reagent B (L-His)	-----	-----	2.50	2.50
 Side Arm				
Reagent D (Enzyme Solution)		-----	0.10	0.10
Reagent C (PRP)	-----	0.10	0.10	0.10
Reagent A (Buffer)	0.20	-----	-----	0.10

Be sure to confirm the stability of the pressure with the flask sealed off before proceeding with the assay. This is to ensure temperature equilibrium and the absence of leaks in the flask.

The enzyme activity is determined by calculation of the rate of production of CO₂ at 37°C.² The reaction rate should be linear for about 20 minutes.

CALCULATIONS:

$$\frac{\text{Units}}{\text{ml L-Histidine Decarboxylase}} = \frac{(\text{C}) (\text{K}) (\text{Dilution Factor})}{\left(22.4 \frac{\text{l}}{\text{mole}}\right) (\text{ml L-Histidine Decarboxylase})}$$

- C = mm of CO₂ gas evolved/minute²
- K = Warburg flask constant³ in $\mu\text{l}/\text{mm}$
- 22.4 l = Volume gas occupies under STP conditions

UNIT DEFINITIONS:

One unit will release 1.0 μmole of CO₂ from L-histidine per minute at pH 4.5 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 77 mM sodium acetate, 83 mM L-histidine, 2 mM pyridoxal 5-phosphate, and 0.4 - 1.0 unit L-histidine decarboxylase.

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REFERENCE:

Umbreit W.W., Burris R.H., and Stauffer, J.F. (1951) in *Manometric Techniques and Tissue Metabolism*, Burgess Publishing Co. Minneapolis, MN

Lawson, A. and Quinn, A.G. (1967) *Biochemical Journal* **105**, 483-490

NOTES:

1. The tests are done in triplicate, since it is common for the flasks to have small leaks.
2. The mm of CO₂ gas evolved (C) is corrected for any temperature and barometric changes (Thermobarometer) during the experiment and also for the Substrate Blank and Enzyme Blank:

$$\text{mm CO}_2 \text{ corrected} = \text{mm CO}_2 \text{ measured Test} - \text{mm CO}_2 \text{ measured for} \\ [\text{Thermobarometer} + \text{Substrate Blank} + \text{Enzyme Blank}]$$

Values of the corrected mm CO₂ produced are plotted versus time. The best straight line is drawn not necessarily through the origin. The slope, C = mm CO₂/time, is obtained.

3. The flask constant, K, is calculated according to the equation:

$$K = \frac{[(V_g) \left(\frac{273}{T} - 2\right) + V_f a]}{P_o}$$

where

P_o = Standard pressure as mm of manometer fluid

V_g = Volume (in milliliters) of gas in flask
and manometer

V_f = Volume (in milliliters) of liquid in flask

T = Absolute temperature

a = Solubility of gas; (for CO₂ at 37°C, a = 0.57)

**Enzymatic Assay of L-HISTIDINE DECARBOXYLASE
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NOTES: (continued)

3. The flask constant, K , must be calculated for each Warburg flask used, as described in Umbreit, W.W., Burris, R.H. and Stauffer, J.F. (1951).
4. This assay is based on the cited references.
5. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.