Enzymatic Assay of L-ORNITHINE DECARBOXYLASE
(EC 4.1.1.17)

PRINCIPLE:

\[
\text{L-Orn} \quad \xrightarrow{\text{Ornithine Decarboxylase}} \quad \text{PRPP} \quad \rightarrow \quad \text{Putrescine + CO}_2
\]

Abbreviations:
L-Orn = L-Ornithine
PRP = Pyridoxal 5-Phosphate

CONDITIONS:  T = 37°C, pH 5.2

METHOD:  Manometric Assay using Warburg Flasks

Reagents:

A. 100 mM Citric Acid and 100 mM Potassium Phosphate Buffer,
   pH 5.2 at 37°C
   (Prepare 100 ml in deionized water using Citric Acid,
    Free Acid, Anhydrous, and
    Potassium Phosphate, Tribasic.
    Adjust the pH to 5.2 at 37°C with 1 M KOH.)

B. 100 mM L-Ornithine Hydrochloride Solution
   (Prepare 50 ml in Reagent A using L-Ornithine
    Hydrochloride.  Adjust the
    pH to 5.2, with 1 M KOH, if necessary.)

C. 10 mM Pyridoxal 5-Phosphate Solution (PRP)
   (Prepare 25 ml in Reagent A using Pyridoxal 5-
    Phosphate.)

D. Ornithine Decarboxylase Enzyme Solution
   (Immediately before use, prepare a solution containing
    2 units/ml of L-Ornithine Decarboxylase in cold
    deionized water.)
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**PROCEDURE:**

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

<table>
<thead>
<tr>
<th>Main Chamber</th>
<th>Thermobarometer Flask</th>
<th>Enzyme Blank</th>
<th>Test(^1) Blank</th>
<th>Substrate Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.80</td>
<td>2.80</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Reagent B (Ornithine HCl)</td>
<td>------</td>
<td>------</td>
<td>2.50</td>
<td>2.50</td>
</tr>
</tbody>
</table>

Side Arm

| Reagent D (Enzyme Solution) | ------ | 0.10 | 0.10 |
| Reagent C (PRP) | ------ | 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 and 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 CO\(_2\) at 37°C.\(^2\) The reaction rate should be linear for about 20 minutes.

**CALCULATIONS:**

\[
\text{Units} = \frac{(C) (K) (\text{Dilution Factor})}{\text{ml Ornithine Decarboxylase}} \times \frac{1}{22.4} \text{ mole l}^2 \text{ (ml Ornithine Decarboxylase)}
\]

C = mm of CO\(_2\) gas evolved/minute\(^2\)
K = Warburg flask constant\(^3\) in μl/mm
22.4 l = Volume gas occupies under STP conditions

**UNIT DEFINITIONS:**

One unit will release 1.0 μmole of CO\(_2\) from L-Ornithine per minute at pH 5.2 at 37°C.

**FINAL ASSAY CONCENTRATIONS:**

In a 3.00 ml reaction mix, the final concentrations are 97 mM citric acid, 97 mM potassium phosphate, 83 mM L-ornithine, 0.33 mM pyridoxal 5-phosphate, and 0.2 unit L-ornithine decarboxylase.
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REFERENCE:


NOTES:

1. The tests are done in triplicate, at least, since it is common for the flasks to have small leaks.

2. The mm of CO\textsubscript{2} gas evolved (C) is corrected for any temperature and barometric changes (Thermobarometer) during the experiment and is also corrected for the Substrate Blank and Enzyme Blanks:

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

Values of the corrected mm CO\textsubscript{2} produced are plotted versus time. The best straight line is drawn not necessarily through the origin. The slope, \( C = \frac{\text{mm CO}_2}{\text{time}} \), is obtained.

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

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

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 CO\textsubscript{2} at 37°C, \( a = 0.57 \))

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 reference.