Enzymatic Assay of L-LYSINE DECARBOXYLASE  
(EC 4.1.1.18)

PRINCIPLE:

\[
\text{L-lysine} \xrightarrow{\text{L-lysine decarboxylase}} \text{cadaverine} + \text{CO}_2
\]

Abbreviation used:  
PRP = Pyridoxal 5-Phosphate

CONDITIONS:  
\( T = 37^\circ C, \text{pH } 6.0 \)

METHOD:  
Manometric Assay using Warburg Flasks

Reagents:

A. 500 mM Sodium Acetate Buffer, pH 6.0 at 37°C  
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate. Adjust to pH 6.0 at 37°C with 1 M HCl.)

B. 100 mM \( \text{L-lysine} \) Solution (\( \text{L-Lys} \))  
(Prepare 50 ml in Reagent A using L-Lysine, Monohydrochloride. Adjust to pH 6.0 at 37°C, if necessary, with either 1 M NaOH or 1 M HCl.)

C. 50 mM Pyridoxal 5-Phosphate Solution (PRP)  
(Prepare 10 ml in Reagent A using Pyridoxal 5-Phosphate.)

D. L-Lysine Decarboxylase Enzyme Solution  
(Immediately before use, prepare a solution containing 2 - 4 units/ml of L-Lysine Decarboxylase in cold Reagent A.)
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PROCEDURE:

Main Chamber

<table>
<thead>
<tr>
<th>Thermobarometer</th>
<th>Enzyme</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flask Blank</td>
<td>Test 1</td>
<td>Blank</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.80</td>
<td>2.80</td>
</tr>
<tr>
<td>Reagent B (L-Lys)</td>
<td>------</td>
<td>------</td>
</tr>
</tbody>
</table>

Side Arm

<table>
<thead>
<tr>
<th>Thermobarometer</th>
<th>Enzyme</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flask Blank</td>
<td>Test 1</td>
<td>Blank</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.20</td>
<td>------</td>
</tr>
<tr>
<td>Reagent C (PRP)</td>
<td>------</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent D (Enzyme Solution)</td>
<td>------</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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. The reaction rate should be linear for about 20 minutes. Obtain the maximum linear rate.

CALCULATIONS:

\[
\text{Units} = \frac{(C)(K)(df)}{ml \text{ L-Lysine Decarboxylase} (22.4 \text{ l mole}^{-1}) (ml \text{ L-Lysine Decarboxylase})}
\]

- \(C\) = mm of CO₂ gas evolved/minute
- \(K\) = Warburg flask constant in μl/mm
- \(df\) = Dilution factor
- 22.4 l = Volume gas occupies under STP conditions

UNIT DEFINITIONS:

One unit will release 1.0 μmole of CO₂ from L-lysine per minute at pH 6.0 at 37°C.

FINAL ASSAY CONCENTRATIONS:
In a 3.00 ml reaction mix, the final concentrations are 500 mM sodium acetate, 83 mM L-lysine, 1.7 mM pyridoxal 5-phosphate, and 0.2 – 0.4 unit L-lysine decarboxylase.
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REFERENCES:


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:

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

Values of the corrected mm CO₂ produced are plotted versus time. The best straight line is drawn not necessarily through the origin. The slope, \( C = \text{mm CO}_2 / \text{time} \), is obtained.

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

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

where

\( P_o = \text{Standard pressure as mm of manometer fluid} \)
\( V_g = \text{Volume (in milliliters) of gas in flask and manometer} \)
\( V_f = \text{Volume (in milliliters) of liquid in flask} \)
\( T = \text{Absolute temperature} \)
\( a = \text{Solubility of gas; (for CO}_2 \text{ at 37°C, a = 0.57)} \)

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).
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NOTES:  (continued)

4. This assay is based on the cited references.

This procedure is for informational purposes.