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, Sigma Prod. No. S-8625. Adjust
   to pH 6.0 at 37°C with 1 M HCl.)

B. 100 mM \( \text{L-Lys} \) Solution (\( \text{L-Lys} \))
   (Prepare 50 ml in Reagent A using \( \text{L-Lys} \),
   Monohydrochloride, Sigma Prod. No. L-5626. 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, Sigma Prod. No. P-9255.)

D. \( \text{L-Lysine Decarboxylase Enzyme Solution} \)
   (Immediately before use, prepare a solution containing
   2 - 4 units/ml of \( \text{L-Lysine Decarboxylase} \) in cold
   Reagent A.)
Enzymatic Assay of L-LYSINE DECARBOXYLASE  
(EC 4.1.1.18)

PROCEDURE:

Main Chamber

<table>
<thead>
<tr>
<th></th>
<th>Flasks</th>
<th>Enzyme</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.80</td>
<td>2.80</td>
<td>0.30</td>
</tr>
<tr>
<td>Reagent B (l-Lys)</td>
<td>------</td>
<td>------</td>
<td>2.50</td>
</tr>
</tbody>
</table>

Side Arm

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.20</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Reagent C (PRP)</td>
<td>------</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent D (Enzyme Solution)</td>
<td>------</td>
<td>0.10</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.² The reaction rate should be linear for about 20 minutes. Obtain the maximum linear rate.

CALCULATIONS:

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

C = mm of CO₂ gas evolved/minute²
K = Warburg flask constant³ in µl/mm
df = Dilution factor
22.4 1 = 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.
Enzymatic Assay of L-LYSINE DECARBOXYLASE  
(EC 4.1.1.18)
Enzymatic Assay of L-LYSINE DECARBOXYLASE  
(EC 4.1.1.18)

NOTES: (continued)

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.