Enzymatic Assay of L-LYSINE DECARBOXYLASE (EC 4.1.1.18)

PRINCIPLE:

$$\text{L-Lysine} \xrightarrow[\text{PRP}]{\text{L-Lysine Decarboxylase}} \text{Cadaverine} + CO_2$$

Abbreviation used:
PRP = Pyridoxal 5-Phosphate

CONDITIONS: T = 37°C, 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 L-Lysine Solution (L-Lys)
(Prepare 50 ml in Reagent A using L-Lysine, 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. 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

	Thermo-barometer Flask	Enzyme Blank	Test[1]	Substrate Blank
Reagent A (Buffer)	2.80	2.80	0.30	0.30
Reagent B (L-Lys)	------	------	2.50	2.50

Side Arm

Reagent A (Buffer)	0.20	------	------	0.10
Reagent C (PRP)	------	0.10	0.10	0.10
Reagent D (Enzyme Solution)		------	0.10	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 CO_2 at 37°C.[2] The reaction rate should be linear for about 20 minutes. Obtain the maximum linear rate.

CALCULATIONS:

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

C = mm of CO_2 gas evolved/minute[2]
K = Warburg flask constant[3] 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 CO_2 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:

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

Sabo, D.L., Boeker, E.A., Byers, B., Waron, H., and Fischer, E.H. (1974) *Biochemistry* **13**, 662-670

NOTES:

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

2. The mm of CO_2 gas evolved (C) is corrected for any temperature and barometric changes (Thermobarometer) during the experiment and also for the Substrate Blank and Enzyme Blank:

   mm $CO_{2\ corrected}$ = mm $CO_{2\ measured}$ Test - mm $CO_{2\ measured}$ for [Thermobarometer + Substrate Blank + Enzyme Blank]

   Values of the corrected mm CO_2 produced are plotted versus time. The best straight line is drawn not necessarily through the origin. The slope, C = mm CO_2/time, is obtained.

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

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

   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 CO_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).

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.