Enzymatic Assay of L-PHENYLALANINE DECARBOXYLASE (EC 4.1.1.53)

PRINCIPLE:

L-Phenylalanine $\xrightarrow[\text{PRP}]{\text{L-Phenylalanine Decarboxylase}}$ Phenylethylamine + CO_2

Abbreviation used:
PRP = Pyridoxal 5-Phosphate

CONDITIONS: T = 37°C, pH 5.5

METHOD: Radiolabelled Stop Reaction

REAGENTS:

A. 200 mM Sodium Phosphate Solution
(Prepare 50 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0876.)

B. 100 mM Citrate Solution
(Prepare 100 ml in deionized water using Citric Acid, Free Acid, Anhydrous, Sigma Prod. No. C-0759.)

C. McIlvane's Buffer, pH 5.5 at 37°C (Buffer)
(Prepare using 50 ml of Reagent A and adjust to pH 5.5 at 37°C with Reagent B.)

D. 3.0 mM L-Phenylalanine Solution
(Prepare 10 ml in Reagent B using L-Phenylalanine, Free Base, Sigma Prod. No. P-2126. Heat (70°C) may be required in order to effect dissolution.)

E. 0.3 mM Pyridoxal 5-Phosphate Solution (PRP)
(Prepare 2 ml in Reagent B using Pyridoxal 5-Phosphate, Sigma Prod. No. P-9255.)

F. ^{14}C[COOH] L-Phenylalanine
(Use ^{14}C[COOH] L-Phenylalanine, 50 - 60 mCi/mmol, 50 µCi/ml.)

G. L-Phenylalanine Reaction Cocktail (L-Phe)
(Prepare by adding 0.05 ml of Reagent E to 2 ml of Reagent C.)

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REAGENTS:

H. 1 M Methylbenzethonium Hydroxide Solution (MBH)
(Use Benzethonium Hydroxide, approximately 1.0 M solution in Methanol, Sigma Prod. No. B-2156)

I. Filter Paper
(1 cm x 2 cm strips of filter paper are prepared using Whatman 3 MM paper. Add 0.050 ml of Reagent G to each strip and allow to air dry. This step is to be carried out in a fume hood.)

J. Vacutainer Tubes
(Use Vacutainer Blood Tubes, 13 x 75 mm, Becton Dickinson.)

K. 25% (w/v) Trichloroacetic Acid Solution (TCA)
(Prepare 10 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, approximately 100% (w/v), Sigma Stock No. 490-10.)

L. L-Phenylalanine Decarboxylase Enzyme Solution
(Immediately before use, prepare a solution containing approximately 0.005 - 0.02 unit/ml of L-Phenylalanine Decarboxylase in cold Reagent C.)

M. Scintillation Cocktail (Scint Fluid)
(Use Sigma-Fluor Universal LSC Cocktail for Aqueous Samples, Sigma Prod. No. S-4273.)

PROCEDURE:

Radiolabelled CO_2 is trapped by absorbing it onto Methylbenzethonium Hydroxide which is present on filter paper located above the reaction mixture in a vacutainer tube.

Pipette (in milliliters) the following reagents into suitable vacutainer tubes.

	Test	Blank	PRP[1]
Reagent G (L-Phe)	0.10	0.10	0.10
Reagent E (PRP)	0.10	0.10	------
Reagent C (Buffer)	------	------	0.10

Equilibrate to 37°C. Then add:

Reagent L (Enz Soln)	0.10	------	0.10
Reagent C (Buffer)	------	0.10	------

PROCEDURE: (continued)

Immediately place Reagent H (Filter Paper) in the top of the vacutainer tube and seal the tubes by replacing the stopper. Incubate for exactly 10 or 20 minutes. Then add by injecting with a syringe and needle through the stopper (**do not remove stopper to add TCA**) (Reagent J):

	Test	Blank	PRP[1]
Reagent K (TCA)	0.40	0.40	0.40

Mix by swirling. Allow to stand for 12-16 hours at room temperature to allow for all evolved CO_2 to be absorbed onto the filter paper.

Remove filter papers from vacutainer tubes and place into scintillation vials containing 7 ml of Reagent L (Scint Fluid). This should be performed in a hood.

Potential DPM (disintegrations per minute) are prepared by pipetting 0.05 ml of Reagent F (L-Phe) onto Reagent H (Filter Paper). Place into scintillation vials containing 7 ml of Reagent L (Scint Fluid).

CALCULATIONS:

$$\text{Potential DPM/}\mu\text{mole} = \frac{\text{DPM of potential}}{\text{Total }\mu\text{moles of L-Phenylalanine}}$$

$$\text{Units/ml enzyme} = \frac{(\text{DPM Test} - \text{DPM Blank})(df)}{(T)(0.1)(\text{Potential DPM/}\mu\text{mole})}$$

DPM = Disintegrations per minute
df = Dilution factor
T = Time (in minutes) of assay as per the Unit Definition
0.1 = Volume (in milliliter) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

UNIT DEFINITION:

One unit will liberate 1.0 µmole of CO_2 from L-phenylalanine per minute at pH 5.5 at 37°C.

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FINAL ASSAY CONCENTRATION:

In a 0.30 ml reaction mix, the final concentrations are 100 mM citric phosphate[3], 0.98 mM L-phenylalanine, 0.1 mM pyridoxal 5-phosphate, and 0.0005 - 0.002 unit L-phenylalanine decarboxylase.

REFERENCE:

Sundaresan, P.R. and Coursin, D.B. (1970) *Methods in Enzymology*, XVIII, Part A, 509-512

NOTES:

1. The enzyme activity is also measured without any Reagent D(PRP) present.
2. This assay is based on the cited reference.
3. This concentration is only an estimate since it is not known what exact volume of sodium phosphate is required to adjust the pH to 5.5 at 37°C.
4. 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.