Enzymatic Assay of L-PHENYLALANINE HYDROXYLASE (EC 1.14.16.1)

PRINCIPLE:

6-MTHP + L-Phenylalanine $\xrightarrow{PAH}$ 6-MDHP + L-Tyrosine

Abbreviations:
6-MTHP = 6-Methyltetrahydropterine
6-MDHP = 6-Methyldihydropterine
PAH = L-Phenylalanine Hydroxylase

CONDITIONS: T = 25°C, pH = 7.2, A_{450nm}, Light Path = 1 cm

METHOD: Colorimetric

REAGENTS:

A. 200 mM Tris HC1 Buffer, pH 7.2 at 25EC
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.2 at 25°C with
1 M HCl.)

B. 4.0 mM L-Phenylalanine Solution (PHE)
(Prepare 30 ml in Reagent A using L-Phenylalanine, Sigma Prod. No. P-2126.)

C. Catalase Enzyme Solution (CAT)
(Prepare 20 ml of a solution containing 4000 units/ml of Catalase, Sigma Stock No. C-100, in cold Reagent B (PHE). **PREPARE FRESH**.)

D. 16.65 mM DL-Dithiothreitol Solution (DTT)
(Prepare 25 ml in deionized water using DL-Dithiothreitol, Sigma Prod. No. D-0632.)

E. 1.33 mM 6-Methyltetrahydropterine Solution (6-MTHP)
(Prepare 10 ml in Reagent D (DTT) using DL-6-Methyl-5,6,7,8-Tetrahydropterine Dihydrochloride, Sigma Prod. No. M-4758. **PREPARE FRESH**.)

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REAGENTS: (continued)

F. 5% (v/v) Trichloroacetic Acid Solution (TCA)
(Prepare 25 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, Sigma Stock No. 490-10.)

G. 20% (v/v) Nitric Acid Solution with 0.05% (w/v) Sodium Nitrite (Nitric Acid)
(Prepare 20 ml in deionized water using Nitric Acid, Aldrich Stock No. 25811-3 and Sodium Nitrite, Sigma Prod. No. S-2252. Dissolve the Sodium Nitrite in deionized water before adding to the Nitric Acid solution.)

H. 0.1% (v/v) Nitrosonaphthol Solution (NNS)
(Prepare 20 ml in Reagent I (NaOH) using 1-Nitroso-2-Naphthol, Sigma Prod. No. N-3765.[1])

I. 100 mM Sodium Hydroxide Solution (NaOH)
(Prepare 50 ml in deionized water using Sodium Hydroxide Solution, 1.0 N, Sigma Stock No. 930-65.)

J. 5.0 mM L-Tyrosine Standard Solution (TYR Std)
(Prepare 10 ml in deionized water using L-Tyrosine Free Base, Sigma Prod. No. T-3754.)

K. Phenylalanine Hydroxylase Enzyme Solution
(Immediately before use, prepare a solution containing 0.2 - 1.0 unit/ml of Phenylalanine Hydroxylase in cold Reagent A.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable containers:

	Test	Blank	Std 1	Std 2	Std 3	Std Blank
Reagent C (CAT)	0.50	0.50	0.50	0.50	0.50	0.50
Reagent K (Enzyme Soln)	0.02	0.02	---	---	---	---
Deionized Water	0.18	0.18	0.17	0.14	0.11	0.20
Reagent J (TYR Std)	---	---	0.03	0.06	0.09	---

Mix by swirling and equilibrate to 25°C. Then add:

Reagent E (6-MTHP)	0.30	---	0.30	0.30	0.30	0.30

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PROCEDURE: (continued)

Immediately mix by swirling, and incubate at 25°C for exactly 8 minutes. Then add:

	Test	Blank	Std 1	Std 2	Std 3	Std Blank
Reagent F (TCA)	1.00	1.00	1.00	1.00	1.00	1.00
Reagent E (6-MTHP)	---	0.30	---	---	---	---
Reagent G (Nitric Acid)	1.00	1.00	1.00	1.00	1.00	1.00
Reagent H (NNS)	1.00	1.00	1.00	1.00	1.00	1.00

Mix by swirling and heat at 55°C for 30 minutes. Cool to 25°C and centrifuge for 3 minutes. Transfer the solutions to suitable cuvettes and record the A_{450nm} for the Test, Blank, Standards and Standard Blank with a suitable spectrophotometer.

Standard Curve:

ΔA_{450nm} Standard = A_{450nm} Standard - A_{450nm} Standard Blank

Prepare a standard curve by plotting the ΔA_{450nm} for the Standards versus micromoles of Tyrosine.

Sample Determination:

ΔA_{450nm} Sample = A_{450nm} Test - A_{450nm} Blank

Determine the total micromoles of L-Tyrosine produced using the Standard curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles L-Tyrosine produced})(df)}{(0.02)\ (8)}$$

df = Dilution factor
8 = Time correction factor (in minutes) as per Unit Definition
0.02 = Volume (in milliliters) of enzyme used in the assay

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

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

UNIT DEFINITION:

One unit will convert 1.0 µmole of L-phenylalanine to L-tyrosine per min at pH 7.2 and 25°C, using DL-methyl-5,6,7,8-tetrahydropterine as cofactor.

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FINAL ASSAY CONCENTRATION:

In a 1.00 ml reaction mix, the final concentration are 104 mM Tris, 2 mM L-phenylalanine, 2000 units catalase, 5.0 mM DL-dithiothreitol, 0.40 mM DL-6-methyl-5,6,7,8-tetrahydropterine and 0.004 - 0.02 unit L-phenylalanine hydroxylase.

REFERENCES:

Bublitz, C. (1969) *Biochim. Biophys. Acta* **191**, 249-256.

NOTES:

1. Dissolve the 1-Nitroso-2-Naphthol in the 100 mM NaOH solution by heating to 100EC for 5 minutes and cooling to 25EC. The solution should be a dark green.

2. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.