Enzymatic Assay of L-PHENYLALANINE DEHYDROGENASE (EC 1.4.1.20)

PRINCIPLE:

L-Phenylalanine + H_2O + ß-NAD ———> Phenylpyruvate + NH_3 + ß-NADH

Abbreviations used:
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form

CONDITIONS: T = 30°C, pH = 10.5, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 250 mM Glycine Buffer, pH 10.5 at 30°C (Gly Buff)
(Prepare 100 ml in deionized water using Glycine, Free Base, Sigma Prod. No. G-7126. Adjust to pH 10.5 at 30°C with 5 M NaOH.)

B. 50 mM Potassium Phosphate, Monobasic Solution
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379.)

C. 50 mM Potassium Phosphate Buffer, pH 7.5 at 30°C (Enz Dil)
(Prepare 50 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Sigma Prod. No. P-5504. Adjust to pH 7.5 at 30°C with Reagent B.)

D. 100 mM L-Phenylalanine Substrate Solution (Phe)
(Prepare 10 ml in deionized water using L-Phenylalanine, Sigma Prod. No. P-2126.)

E. 30 mM ß-Nicotinamide Adenine Dinucleotide, Oxidized Form Solution (ß-NAD)
(Dissolve the contents of one 20 mg vial of ß-Nicotinamide Adenine Dinucleotide, Sigma Stock No. 260-120 in the appropriate volume of deionized water or prepare 10 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-

1511.)

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REAGENTS: (continued)

F. L-Phenylalanine Dehydrogenase Enzyme Solution (Prepare a solution containing 3-10 units of L-Phenylalanine Dehydrogenase in cold Reagent C. Store at 4°C. Immediately before use, dilute to 0.10 - 0.20 unit/ml in cold Reagent A.)

PROCEDURE:

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

	Test	Blank
Reagent A (Gly Buff)	2.30	2.30
Reagent D (Phe)	0.30	0.30
Reagent E (ß-NAD)	0.30	0.30

Mix by inversion and equilibrate to 30°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent F (Enzyme Solution)	0.10	------
Reagent C (Enz Dil)	------	0.10

Immediately mix by inversion and record the increase in A_{340nm} for approximately 5 minutes. Obtain the $r\ A_{340nm}$/min using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r\,A_{340nm}/\text{min Test} - r\,A_{340nm}/\text{min Blank})(3)(df)}{(6.22)(0.1)}$$

3 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
0.1 = Volume (in milliliter) of enzyme used

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

CALCULATIONS: (continued)

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

UNIT DEFINITION:

One unit will oxidize 1.0 µmole of L-phenylalanine per minute at pH 10.5 at 30°C in the presence of ß-NAD.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 192 mM glycine, 10 mM L-phenylalanine, 3 mM ß-nicotinamide adenine dinucleotide, 1.7 mM potassium phosphate and 0.01 - 0.02 unit L-phenylalanine dehydrogenase.

REFERENCE:

Asano, Y., Nakazawa, A. and Endo, K. (1987) *Journal of Biological Chemistry* **262**, 10346-10354

NOTES:

1. This assay is based on the cited reference.

2. 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.