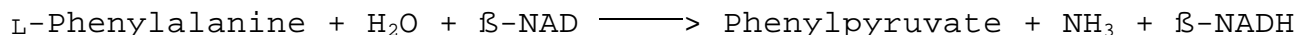


**Enzymatic Assay of L-PHENYLALANINE DEHYDROGENASE  
(EC 1.4.1.20)**

**PRINCIPLE:**



Abbreviations used:

$\beta$ -NAD =  $\beta$ -Nicotinamide Adenine Dinucleotide, Oxidized Form

$\beta$ -NADH =  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form

**CONDITIONS:** T = 30°C, pH = 10.5,  $A_{340\text{nm}}$ , 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  $\beta$ -Nicotinamide Adenine Dinucleotide, Oxidized Form Solution ( $\beta$ -NAD)  
(Dissolve the contents of one 20 mg vial of  $\beta$ -Nicotinamide Adenine Dinucleotide, Sigma Stock No. 260-120 in the appropriate volume of deionized water or prepare 10 ml in deionized water using  $\beta$ -Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-

1511.)

**Enzymatic Assay of L-PHENYLALANINE DEHYDROGENASE  
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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:

	<u>Test</u>	<u>Blank</u>
Reagent A (Gly Buff)	2.30	2.30
Reagent D (Phe)	0.30	0.30
Reagent E (β-NAD)	0.30	0.30

Mix by inversion and equilibrate to 30°C. Monitor the  
A<sub>340nm</sub> 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<sub>340nm</sub> for approximately 5 minutes. Obtain the r A<sub>340nm</sub>/min  
using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

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

3 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of β-NADH at 340  
nm

0.1 = Volume (in milliliter) of enzyme used

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

**Enzymatic Assay of L-PHENYLALANINE DEHYDROGENASE  
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**CALCULATIONS:** (continued)

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

**UNIT DEFINITION:**

One unit will oxidize 1.0  $\mu$ mole of L-phenylalanine per minute at pH 10.5 at 30°C in the presence of  $\beta$ -NAD.

**FINAL ASSAY CONCENTRATION:**

In a 3.00 ml reaction mix, the final concentrations are 192 mM glycine, 10 mM L-phenylalanine, 3 mM  $\beta$ -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.**