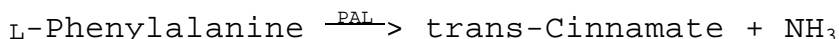


**Enzymatic Assay of PHENYLALANINE AMMONIA-LYASE  
(EC 4.3.1.5)  
L-Phenylalanine as a Substrate**

**PRINCIPLE:**



Abbreviation used:

PAL = Phenylalanine Ammonia-Lyase

**CONDITIONS:** T = 30°C, pH = 8.5, A<sub>270nm</sub>, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

- A. 150 mM Tris HCl Buffer, pH 8.5 at 30°C  
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.5 at 30°C with 1 M HCl.)
- B. 3 mM L-Phenylalanine Solution (PHE)  
(Prepare 25 ml in Reagent A using L-Phenylalanine, Sigma Prod. No. P-2126. Heat gently to dissolve.)
- C. Phenylalanine Ammonia-Lyase (PAL)  
(Immediately before use, prepare a solution containing 0.03 - 0.15 unit/ml of Phenylalanine-Lyase in Reagent A.)

**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Reagent B (PHE)	2.00	2.00
Deionized Water	0.90	0.90

Mix by inversion and equilibrate to 30°C. Then add:

Reagent C (PAL)	0.10	-----
Reagent A (Buffer)	-----	0.10

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**PROCEDURE:** (continued)

Immediately mix by inversion and record the increase in  $A_{270\text{nm}}$  for approximately 5 minutes. Obtain the  $r A_{270\text{nm}}$ /minute using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(r A_{270\text{nm}}/\text{min Test} - r A_{270\text{nm}}/\text{min Blank})(3)(\text{df})}{(19.73)(0.1)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

19.73 = Millimolar extinction coefficient<sup>1</sup> of trans-cinnamate  
at 270nm

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

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

**UNIT DEFINITION:**

One unit will deaminate 1.0  $\mu\text{mole}$  of L-phenylalanine to trans-cinnamate and  $\text{NH}_3$  per minute at pH 8.5 at 30°C.

**FINAL ASSAY CONCENTRATIONS:**

In a 3.00 ml reaction mix, the final concentrations are 150 mM Tris, 2 mM L-phenylalanine, and 0.003 - 0.015 unit phenylalanine ammonia-lyase.

**REFERENCES:**

Hodgins, D.S. (1971) *Journal of Biological Chemistry* **246**, 2977-2985

Havir, E.A. and Hanson, K.R. (1970) *Methods in Enzymology*, XVIIIA, 575-581

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**NOTES:**

1. This value has been determined experimentally by Sigma.
2. This assay is based on the cited references.
3. 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.**