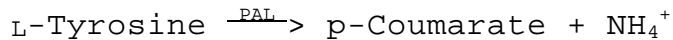


**Enzymatic Assay of PHENYLALANINE AMMONIA-LYASE  
(EC 4.3.1.5)  
L-Tyrosine as Substrate**

**PRINCIPLE:**



Abbreviation used:

PAL = Phenylalanine Ammonia-Lyase

**CONDITIONS:** T = 30°C, pH = 8.5, A<sub>286nm</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-Tyrosine Solution (TYR)  
(Prepare 25 ml in Reagent A using L-Tyrosine, Free Base, Sigma Prod. No. T-3754. Heat gently to dissolve.)
- C. Phenylalanine Ammonia-Lyase Enzyme Solution (PAL)  
(Immediately before use, prepare a solution containing 0.5 - 1.0 unit/ml of Phenylalanine Ammonia-Lyase in cold Reagent A.)

**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Reagent B (TYR)	2.00	2.00
Deionized Water	0.95	0.95

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**PROCEDURE:** (continued)

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

	<u>Test</u>	<u>Blank</u>
Reagent C (PAL)	0.05	-----
Reagent A (Buffer)	-----	0.05

Immediately mix by inversion and record the increase in  $A_{286\text{nm}}$  for approximately 5 minutes. Obtain the  $r A_{286\text{nm}}$ /minute using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(r A_{286\text{nm}}/\text{min Test} - r A_{286\text{nm}}/\text{min Blank})(3)(\text{df})}{(18.5)(0.05)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

18.5 = Millimolar extinction coefficient of p-coumaric acid<sup>1</sup>

at 286nm

0.05 = Volume (in milliliter) of enzyme used

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

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

**UNIT DEFINITION:**

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

**FINAL ASSAY CONCENTRATION:**

In a 3.00 ml reaction mix, the final concentrations are 103 mM Tris, 2 mM L-tyrosine, and 0.025 - 0.050 unit phenylalanine ammonia-lyase.

**REFERENCE:**

Fritz, R.R., Hodgins, D.S., and Abell, C.W. (1976) *Journal of Biological Chemistry* **251**, 4646-4650

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**NOTES:**

1. The millimolar extinction coefficient was determined experimentally by Sigma.
2. This assay is based on the cited reference.

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