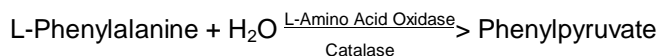




Product Information

SIGMA QUALITY CONTROL TEST PROCEDURE
Enzymatic Assay of L-AMINO ACID OXIDASE
(EC 1.4.3.2)

PRINCIPLE:



CONDITIONS: T = 37°C, pH = 6.5, A_{308nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 200 mM Sodium Phosphate Buffer, pH 6.5 at 37°C
(Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751. Adjust to pH 6.5 at 37°C with 1 M NaOH.)
- B. 10 mM L-Phenylalanine Solution (L-Phe)
(Prepare 10 ml in deionized water using L-Phenylalanine, Sigma Prod. No. P-2126.)
- C. 2000 mM Sodium Arsenate Solution (Arsenate)
(Prepare 20 ml in Reagent A using Arsenic Acid, Sodium Salt, Sigma Prod. No. A-6756.)
- D. 2000 mM Boric Acid Solution, pH 6.5 at 37°C (Boric Acid)
(Prepare 20 ml in Reagent C using Boric Acid, Sigma Prod. No. B-0252. Adjust to pH 6.5 at 37°C with 5 M HCl.)
- E. Catalase Enzyme Solution (Catalase)
(Immediately before use, prepare a solution containing 60,000 units/ml in cold deionized water using Catalase, Sigma Stock No. C-40.)
- F. L-Amino Acid Oxidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.5 - 1.0 unit/ml of L-Amino Acid Oxidase in cold deionized water.)

**Enzymatic Assay of L-AMINO ACID OXIDASE
(EC 1.4.3.2)**

PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

Reagent A (Buffer)	11.70
Reagent B (L-Phe)	3.00
Reagent D (Boric Acid)	14.00

Mix by stirring and adjust to pH 6.5 at 37°C with 1 M HCl or 1 M NaOH, if necessary. Pipette (in milliliters) the following reagents into suitable cuvettes:

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	2.87	2.87
Reagent E (Catalase)	0.03	0.03

Mix by inversion and equilibrate to 37°C. Monitor the A_{308nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

	<u>Test</u>	<u>Blank</u>
Reagent F (Enzyme Solution)	0.10	-----
Deionized Water	-----	0.10

Immediately mix by inversion and record the increase in A_{308nm} for approximately 10 minutes. Obtain the $\Delta A_{308nm}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{308nm}/\text{min Test} - \Delta A_{308nm}/\text{min Blank}) (3) (df)}{(5.00) (0.1)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

5.00 = Millimolar extinction coefficient of the phenylpyruvate keto borate complex at 308 nm

0.1 = Volume (in milliliter) of enzyme

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

**Enzymatic Assay of L-AMINO ACID OXIDASE
(EC 1.4.3.2)**

PROCEDURE: (continued)

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

UNIT DEFINITION:

One unit will oxidatively deaminate 1.0 μ mole of L-phenylalanine per minute at pH 6.5 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 171 mM sodium phosphate, 1.0 mM phenylalanine, 933 mM sodium arsenate, 933 mM boric acid, 1800 units catalase and 0.05 - 0.1 unit L-amino acid oxidase.

REFERENCE:

Wellner, D. and Lichtenber, L. A. (1971) *Methods in Enzymology*, XVII B, 593-596

Knox, W. E. and Pitt, B. M. (1957) *Journal of Biological Chemistry* **225**, 675-688

NOTES:

1. Catalase Unit Definition: One unit will decompose 1.0 μ mole of H₂O₂ per minute at pH 7.0 at 25°C, while the H₂O₂ concentration falls from 10.3 to 9.2 mM.
2. This assay is based on the cited references.
3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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