SIGMA QUALITY CONTROL TEST PROCEDURE

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

ProductInformation

PRINCIPLE:

$$\text{L-Phenylalanine} + H_2O \xrightarrow[\text{Catalase}]{\text{L-Amino Acid Oxidase}} \text{Phenylpyruvate}$$

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.)

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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:

	Test	Blank
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:

	Test	Blank
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 ΔA_{308nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

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

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

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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 µmole 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 µmole of H_2O_2 per minute at pH 7.0 at 25°C, while the H_2O_2 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.