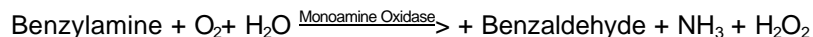


**Enzymatic Assay of PLASMA AMINE OXIDASE
(EC 1.4.3.6)**

PRINCIPLE:



CONDITIONS: T = 25°C, pH = 7.4, $A_{250\text{nm}}$, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 200 mM Potassium Phosphate Buffer, pH 7.4 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate Monobasic, Sigma Prod. No. P-5379. Adjust to pH 7.4 at 25°C with 1 M NaOH.)
- B. 1 M Sulfuric Acid Solution (H_2SO_4)
(Prepare 10 ml in deionized water using Sulfuric Acid, Sigma Prod. No. S-1526.)
- C. 100 mM Benzylamine Sulfate Solution
(Prepare 100 ml in deionized water. Facilitate solubilization by first dissolving into 5 ml of Reagent B using Benzylamine, Hydrochloride, Sigma Prod. No. B-5136. Adjust to pH 7.4 with 1 M NaOH.)
- D. Plasma Amine Oxidase Enzyme Solution
(Immediately before use, prepare a solution containing approximately 0.15 - 0.20 units/ml of Plasma Amine Oxidase in cold Reagent A.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.80	2.80
Reagent C (Benzylamine Sulfate) 0.10	0.10	

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PROCEDURE: (continued)

Mix by inversion and equilibrate to 25°C. Monitor the $A_{250\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

	<u>Test</u>	<u>Blank</u>
Reagent D (Enzyme Solution)	0.10	-----

Immediately mix by inversion and record the increase in $A_{250\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{250\text{nm}}$ /minute by using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{250\text{nm}}/\text{min Test} - \Delta A_{250\text{nm}}/\text{min Blank})(3)(\text{df})}{(11.3)(0.1)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

11.3 = Difference between the millimolar extinction coefficients of benzylamine and benzaldehyde at 250 nm

0.1 = 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 oxidize 1.0 micromole of benzylamine to benzaldehyde per minute at pH 7.4 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 187 mM potassium phosphate buffer, 3.33 mM benzylamine sulfate and 0.015 - 0.02 unit plasma amine oxidase.

REFERENCES:

Tabor, C.W., Tabor, H. and Rosenthal, S.M. (1954) *J. Biol. Chem.* **208**, 645.

Buffoni, F. and Blaschko, H. (1964) *Proc. Roy. Soc.* **B161**, 153.

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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.