Enzymatic Assay of PLASMA AMINE OXIDASE  
(EC 1.4.3.6)

**PRINCIPLE:**

Benzylamine $+ \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{Monoamine Oxidase}}$ + Benzaldehyde + $\text{NH}_3 + \text{H}_2\text{O}_2$

**CONDITIONS:**  $T = 25^\circ\text{C}$, $\text{pH} = 7.4$, $A_{250nm}$, 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.  
   Adjust to pH 7.4 at 25°C with 1 M NaOH.)

B. 1 M Sulfuric Acid Solution ($\text{H}_2\text{SO}_4$)  
   (Prepare 10 ml in deionized water using Sulfuric Acid.)

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.  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:

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.80</td>
</tr>
<tr>
<td>Reagent C (Benzylamine Sulfate)</td>
<td>0.10</td>
</tr>
</tbody>
</table>
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PROCEDURE: (continued)

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent D (Enzyme Solution)</td>
<td>0.10</td>
<td>------</td>
</tr>
</tbody>
</table>

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

CALCULATIONS:

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

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

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

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:


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NOTES:

1. This assay is based on the cited reference.

2. Where our Product or Stock numbers are specified, equivalent reagents may be substituted.

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