Enzymatic Assay of D-AMINO ACID OXIDASE
(EC 1.4.3.3)

PRINCIPLE:

\[ \text{D-Alanine} + \text{O}_2 \xrightarrow{\text{D-Amino Acid Oxidase}} \text{Pyruvate} + \text{NH}_3 \]
\[ \xrightarrow{\text{Catalase}} \]

\[ \text{Pyruvate} + \beta\text{-NADH} \xrightarrow{\text{Lactic Acid Dehydrogenase}} \text{Lactate} + \beta\text{-NAD} \]

Abbreviations used:
\( \beta\text{-NADH} = \beta\text{-Nicotinamide Adenine Dinucleotide, Reduced Form} \)
\( \beta\text{-NAD} = \beta\text{-Nicotinamide Adenine Dinucleotide, Oxidized Form} \)

CONDITIONS: \( T = 25^\circ\text{C}, \text{pH} = 8.3, A_{340\text{nm}}, \) Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 200 mM Tris HCl Buffer, pH 8.3 at 25°C.
   (Prepare 100 ml in deionized water using Trizma Base, Adjust to pH 8.3 at 25°C with 1 M HCl. Bubble oxygen through the buffer for 5 minutes immediately before use.)

B. 224 mM D-Alanine Solution
   (Prepare 5 ml in deionized water using D-Alanine)

C. 6.4 mM \( \beta\text{-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (\( \beta\text{-NADH} \))} \)
   (Dissolve the contents of one 5 mg vial of \( \beta\text{-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt,} \)
   in the appropriate volume of Reagent A. PREPARE FRESH.)

D. Catalase Enzyme Solution
   (Immediately before use, prepare a solution containing 600 units/ml in cold deionized water using Catalase)
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REAGENTS:  (continued)

E. Lactic Dehydrogenase Enzyme Solution (LDH)  
(Immediately before use, prepare a solution containing 400 units/ml in cold deionized water using \( \alpha \)-Lactic Dehydrogenase.)

F. 3.6 M Ammonium Sulfate Solution, pH 6.5 at 25°C (Enz Dil)  
(Prepare 25 ml in deionized water using Ammonium Sulfate. Adjust to pH 6.5 at 25°C with 5 M \( \text{NH}_4 \text{OH} \).)

G. \( \alpha \)-Amino Acid Oxidase Enzyme Solution (\( \alpha \)-AAO)  
(Immediately before use, prepare a solution containing 0.4 - 0.8 unit/ml of \( \alpha \)-Amino Acid Oxidase in cold Reagent F.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
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</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.25</td>
<td>2.25</td>
</tr>
<tr>
<td>Reagent B (( \alpha )-Alanine Solution)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Reagent C (( \beta )-NADH)</td>
<td>0.050</td>
<td>0.050</td>
</tr>
<tr>
<td>Reagent D (Catalase)</td>
<td>0.050</td>
<td>0.050</td>
</tr>
<tr>
<td>Reagent E (LDH)</td>
<td>0.050</td>
<td>0.050</td>
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</tbody>
</table>

Mix by inversion and equilibrate to 25°C. Monitor the \( A_{340\text{nm}} \) until constant, using a suitably thermostatted spectrophotometer. Then add:

<p>| | | |</p>
<table>
<thead>
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<tbody>
<tr>
<td>Deionized Water</td>
<td>------</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent G (( \alpha )-AAO)</td>
<td>0.10</td>
<td>------</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the decrease in \( A_{340\text{nm}} \) for approximately 5 minutes. Obtain the \( r A_{340\text{nm}}/\text{minute} \) using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

\[
\text{Units/mg enzyme} = \frac{r A_{340\text{nm}}/\text{min Test} - r A_{340\text{nm}}/\text{min Blank}}{(6.22) (\text{mg enzyme/ml RM})}
\]

6.22 = Millimolar extinction coefficient of \( \beta \)-NADH at 340
nm
RM = Reaction Mix
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UNIT DEFINITION:

One unit will oxidatively deaminate 1.0 µmole of d-alanine to pyruvate per minute at pH 8.3 at 25°C, in the presence of catalase. (This is equivalent to an O₂ uptake of approximately 335 µl in 30 minutes.)

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 153 mM Tris, 37 mM d-alanine, 0.11 mM β-NADH, 30 units catalase, 20 units lactic acid dehydrogenase, 120 mM ammonium sulfate, and 0.04 – 0.08 unit d-amino acid oxidase.

REFERENCES:


NOTES:

1. Catalase Unit Definition: One unit will decompose 1.0 µmole of H₂O₂ per minute at pH 7.0 at 25°C, while the H₂O₂ concentration falls from 10.3 to 9.2 mM.

2. Lactic Dehydrogenase Unit Definition: One unit will reduce 1.0 µmole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.

3. All product and stock numbers, unless otherwise indicated, are OUR product and stock numbers.

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