Enzymatic Assay of ASPARAGINASE
(EC 3.5.1.1)

PRINCIPLE:
L-Asparagine + H₂O $\xrightarrow{\text{Asparaginase}}$ L-Aspartate + NH₃

CONDITIONS: T = 37°C, pH = 8.6, A₄₃₆nm, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 50 mM Tris Buffer, pH 8.6 at 37°C
   (Prepare 100 ml in deionized water using Trizma Base. Adjust to pH 8.6 at 37°C with 1 M HCl.)

B. 189 mM L-Asparagine Solution
   (Prepare 10 ml in deionized water using L-Asparagine, Anhydrous.)

C. 6 mM Ammonium Sulfate Standard Solution ((NH₄)₂SO₄ Std)
   (Prepare 100 ml deionized water using Ammonium Sulfate, Grade I.)

D. 1.5 M Trichloroacetic Acid (TCA)
   (Prepare 10 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution)

E. Ammonia Color Reagent
   (Use Nessler's Reagent.)

F. Asparaginase Enzyme Solution
   (Immediately before use, prepare a solution containing 2.0 - 4.0 units/ml of Asparaginase in cold deionized water.)
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(EC 3.5.1.1)

PROCEDURES:

Step 1:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Reagent B (L-Asparagine Soln)</td>
<td>0.10</td>
<td>0.10</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Reagent C ((NH₄)₂SO₄ Std)</td>
<td>---</td>
<td>---</td>
<td>0.25</td>
<td>0.50</td>
<td>1.00</td>
<td>---</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>0.90</td>
<td>0.90</td>
<td>0.85</td>
<td>0.60</td>
<td>0.10</td>
<td>1.10</td>
</tr>
</tbody>
</table>

Equilibrate to 37°C. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent F (Enzyme Solution)</td>
<td>0.10</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and incubate at 37°C for 30 minutes. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent D (TCA)</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent F (Enzyme Solution)</td>
<td>---</td>
<td>0.10</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

Mix by inversion. Centrifuge for 2 minutes to clarify.

Step 2:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>4.30</td>
<td>4.30</td>
<td>4.30</td>
<td>4.30</td>
<td>4.30</td>
<td>4.30</td>
</tr>
<tr>
<td>Supernatant (from Step 1)</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Reagent E (Ammonia Color Reagent)</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and after 1 minute record the \( A_{436nm} \) for Standards, Tests, and Blanks.
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(EC 3.5.1.1)

CALCULATIONS:

Standard Curve:

\[ r \ A_{436\text{nm}} \text{ Standard} = A_{436\text{nm}} \text{ Standard} - A_{436\text{nm}} \text{ Standard Blank} \]

Prepare a standard curve by plotting the \( r \ A_{436\text{nm}} \) of the Standard versus Ammonia (\( \text{NH}_3 \)) concentration. Note that 1 mole of Ammonium Sulfate corresponds to 2 moles of Ammonia, therefore a 6 mM Ammonium Sulfate standard is equivalent to a 12 mM ammonium standard.

Sample Determination:

\[ r \ A_{436\text{nm}} \text{ Test} = A_{436\text{nm}} \text{ Test} - A_{436\text{nm}} \text{ Test Blank} \]

Determine the \( \mu \)moles of \( \text{NH}_3 \) liberated using the standard curve.

\[
\text{Units/ml enzyme} = \frac{(\mu \text{mole of } \text{NH}_3 \text{ liberated})(2.20)}{(0.2)(30)(0.1)}
\]

2.20 = Volume of Step 1
0.2 = Volume of Step 1 used in Step 2
30 = Time of assay in minutes
0.1 = Volume (in milliliters) 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 liberate 1.0 \( \mu \)mole of ammonia from L-asparagine per minute at pH 8.6 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 2.20 ml reaction mix, the final concentrations are, 23 mM Tris, 8.6 mM L-asparagine and 0.2 - 0.4 units of asparaginase.
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REFERENCES:


NOTES:

1. This assay is based on the cited reference.