Enzymatic Assay of UREASE
(EC 3.5.1.5)

PRINCIPLE:

\[ \text{Urea} + \text{H}_2\text{O} \xrightarrow{\text{Urease}} \text{CO}_2 + 2\text{NH}_3 \]

CONDITIONS:  \( T = 30^\circ\text{C}, \text{pH} = 8.2, A_{480\text{nm}}, \) Light Path = 1 cm

METHOD:  Spectrophotometric Stop Rate Determination

REAGENTS:

A. 10 mM Potassium Phosphate Buffer, pH 8.2 at 30°C with 10 mM Lithium Chloride and 1 mM Ethylenediaminetetraacetic Acid.
   (Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Lithium Chloride, Anhydrous, and Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Hydrate. Adjust to pH 8.2 at 30°C with 1 M HCl.)

B. 66 mM Urea Solution.
   (Prepare 25 ml in Reagent A using Urea.)

C. 0.4% (w/v) Ficoll Solution
   (Prepare 20 ml in deionized water using Ficoll, Type 400-DL.)

D. Nessler's Color Reagent (NCR)
   (Prepare 75 ml using 15 ml Aldrich Nessler's Reagent, Aldrich, 15 ml Reagent C, and 45 ml deionized water.)

E. 2.50 mM Ammonia Standard Solution (Amm Std)
   (Prepare 50 ml in deionized water using Ammonium Sulfate.)

F. Urease Enzyme Solution
   (Immediately before use, prepare a solution containing 100 - 150 units/ml of Urease in cold Reagent A.)
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**PROCEDURE:**

Pipette (in milliliters) the following reagents into a suitable container:

<table>
<thead>
<tr>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reagent B (Urea Solution)</strong></td>
</tr>
</tbody>
</table>

Incubate at 30°C. Then at time zero add:

| **Reagent F (Enzyme Solution)** | 0.025 |

Immediately mix by inversion and remove 1.0 ml aliquot from the Test solution. Transfer to a suitable container containing 5.0 ml of Reagent D (NCR) and mix by inversion. This is to be used as the Reagent Blank. Continue to incubate at 30°C. Then at 2, 4, and 6 minutes (time increments), transfer 1.0 ml Test solution into suitable containers containing 5.0 ml of Reagent D (NCR) and mix by inversion. Transfer the solutions to suitable cuvets and record the $A_{480nm}$ for the Reagent Blank and Tests using a suitable spectrophotometer.

**COLORIMETRIC:**

Standards:

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

<table>
<thead>
<tr>
<th>Std Blank</th>
<th>Std1</th>
<th>Std2</th>
<th>Std 3</th>
<th>Std 4</th>
<th>Std 5</th>
<th>Std 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized water</td>
<td>1.00</td>
<td>0.90</td>
<td>0.80</td>
<td>0.60</td>
<td>0.40</td>
<td>0.20</td>
</tr>
<tr>
<td>Reagent E (Amm Std)</td>
<td>----</td>
<td>0.10</td>
<td>0.20</td>
<td>0.40</td>
<td>0.60</td>
<td>0.80</td>
</tr>
<tr>
<td>Reagent D (NCR)</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
</tbody>
</table>

Mix by inversion. Transfer to suitable cuvets, measure and record the $A_{480nm}$ for the Standard Blank and Standards.

**CALCULATIONS:**

\[
\begin{align*}
\text{r } A_{480nm} \text{ Standard} &= A_{480nm} \text{ Standard} - A_{480nm} \text{ Standard Blank} \\
\text{Plot } \mu\text{moles of NH}_3 &\text{ vs r } A_{480nm} \text{ to obtain } r \frac{A_{480nm}}{\mu\text{mole NH}_3} \\
\text{r } A_{480nm} \text{ Test} &= A_{480nm} \text{ Test} - A_{480nm} \text{ Reagent Blank} \\
\text{r } A_{480nm} \text{ Test/min} &= \frac{r A_{480nm} \text{ Test}}{\text{Time increment (in minutes)}}
\end{align*}
\]
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CALCULATIONS: (continued)

\[
\text{Units/g enzyme} = \frac{r_{A_{480\text{nm}}}}{(r_{A_{480\text{nm}}}/\mu\text{mole NH}_3)(\text{mg enzyme/ml RM})}
\]

RM = Reaction mixture
1000 = Conversion factor for converting milligrams to grams

UNIT DEFINITION:

One unit will liberate 1.0 µmole of NH\(_3\) from urea per minute at pH 8.2 at 30°C.

FINAL ASSAY CONDITIONS:

In a 10.025 ml reaction mixture the final concentrations are 10 mM potassium phosphate, 10 mM lithium chloride, 1 mM ethylenediaminetetraacetic acid, 66 mM urea and 2.5-3.75 units urease.

This procedure is for informational purposes.