Enzymatic Assay of ARGINASE
(EC 3.5.3.1)

PRINCIPLE:

\[ \text{L-Arginine} + \text{H}_2\text{O} \xrightarrow{\text{Arginase}} \text{L-Ornithine} + \text{Urea} \]

CONDITIONS: \( T = 37^\circ\text{C}, \text{pH} = 9.5, A_{535\text{nm}}, \text{Light path} = 1 \text{ cm} \)

METHOD: Colorimetric

REAGENTS:

A. 50 mM Manganese Maleate Activation Buffer, pH 7.0 at 37°C
   The following components are made separately:
   1. 100 mM Manganese Sulfate Solution
      (Prepare 60 ml in deionized water using Manganese Sulfate. 
       PREPARE FRESH.)
   2. 125 mM Maleic Acid Solution, pH 8.0 at 37°C
      (Prepare 50 ml in deionized water using Maleic Acid. Adjust
      the pH to 8.0 at 37°C using 2 M NaOH.)
      Combine 50 ml of Component 1 and 40 ml of Component 2 and mix. Equilibrate to 37°C
      and adjust to pH 7.0 using 0.1 M HCl. Dilute to a final volume of 100 ml with deionized
      water.

B. 713 mM L-Arginine Substrate Solution
   (Prepare 50 ml in deionized water using L-Arginine, Free Base. 
   Equilibrate to 37°C and adjust to pH 9.5 using 5 M HCl. The L-Arginine will dissolve upon the 
   addition of the HCl. PREPARE FRESH.)

C. Arginase Enzyme Solution
   (Prepare a solution containing 40-60 units/ml in Reagent A. Activate the enzyme by
   incubating for 4 hours at 37°C. Dilute 0.1 ml of activated enzyme to 50 ml using deionized
   water, immediately prior to use.)
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REAGENTS: (continued)

D. 20 mM Urea Stock Solution, pH 9.5.
   (Prepare 50 ml in deionized water using Urea. Adjust to pH 9.5
   using 0.1 N NaOH.)

E. 4 mM Urea Standard Solution, pH 9.5.
   (Prepare 10 ml by diluting 2 ml of Reagent D to 10 ml with deionized water.)

F. BUN Acid-Color Reagent (BUN)
   (Urea Nitrogen Kit, Sigma Stock No. 535-A. Immediately before use, add 60 ml of BUN Acid
   Reagent, to 40 ml of BUN Color Reagent, and mix.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blk</th>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Std 4</th>
<th>Std 5</th>
<th>Std Blk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C</td>
<td>0.30</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
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<tr>
<td>(Enz Soln)</td>
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<td></td>
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<tr>
<td>Reagent E</td>
<td></td>
<td></td>
<td>0.03</td>
<td>0.05</td>
<td>0.10</td>
<td>0.20</td>
<td>0.30</td>
<td>---</td>
</tr>
<tr>
<td>(Urea Standard Soln)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Deionized Water</td>
<td>0.30</td>
<td>0.30</td>
<td>0.57</td>
<td>0.55</td>
<td>0.50</td>
<td>0.40</td>
<td>0.30</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 37°C. Then add:

Reagent B (Substrate Soln) | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40

Mix by inversion and incubate at 37°C for exactly 30 minutes. Then add:

Reagent F (BUN) | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 | 8.00
Reagent C (Enz Soln) |      | 0.30 | ---  | ---  | ---  | ---  | ---  | ---

Mix by inversion and place all the vials in a boiling water bath for 12 minutes. Remove and place the vials in
an ice bath for 3 minutes. Transfer the solutions to suitable cuvettes and read the absorbance at 535 nm for
each of the vials using deionized water as a reference.
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CALCULATIONS:

Standard Curve:

\[ r \ A_{535\text{nm}} \text{ Standard} = A_{535\text{nm}} \text{ Standard} - A_{535\text{nm}} \text{ Standard blank} \]

Plot the \( r \ A_{535\text{nm}} \) of the Standards vs µmoles of urea

Sample Determination:

\[ r \ A_{535\text{nm}} \text{ Sample} = A_{535\text{nm}} \text{ Test} - A_{535\text{nm}} \text{ Test Blank} \]

Determine the µmoles of urea liberated using the Standard Curve.

\[
\text{Units/ml enzyme} = \frac{(\mu\text{moles of urea liberated})(df)}{(30)(0.3)}
\]

\( df = \) Dilution factor
\( 30 = \) Time of assay in minutes
\( 0.3 = \) Volume (in milliliter) of enzyme used

\[
\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}
\]

\[
\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}
\]

FINAL ASSAY CONCENTRATION:

In a 1.00 ml reaction mix, the final concentrations are 0.03 mM manganese malate, 285 mM L-arginine and 0.024 - 0.036 unit arginase.

UNIT DEFINITION:

One unit will convert 1.0 micromole of L-arginine to ornithine and urea per minute at pH 9.5 at 37°C.

NOTES:

This procedure is for informational purposes.