Enzymatic Assay of UREASE (ATP-Hydrolyzing)  
(EC 3.5.1.45)

PRINCIPLE:

\[
\text{Urea} + \text{ATP} \xrightarrow{\text{UA}} 2\text{NH}_3 + \text{CO}_2 + \text{ADP} + \text{P}_i \\
\text{PEP} + \text{ADP} \xrightarrow{\text{PK}} \text{Pyruvate} + \text{ATP} \\
\text{Pyruvate} + \text{NADH} \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}
\]

Abbreviations used:
ATP = Adenosine 5'-Triphosphate  
UA = Urease, ATP-Hydrolyzing  
ADP = Adenosine 5'-Diphosphate  
PEP = Phospho(enol)pyruvate  
PK = Pyruvate Kinase  
NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form  
LDH = Lactic Dehydrogenase  
NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form  
P\text{i} = \text{Inorganic Phosphate}

CONDITIONS:  \( T = 30^\circ\text{C}, \  \text{pH} = 8.0, \ A_{340\text{nm}}, \ \text{Light path} = 1.0 \ \text{cm} \)

METHOD:  Continuous Spectrophotometric Rate Determination

REAGENTS:

A.  100 mM Tris HCl Buffer, pH 8.0 at 30°C  
(Prepare 100 ml in deionized water using Trizma Base.  
Adjust to pH 8.0 at 30°C with 1 M HCl.)

B.  11 mM Adenosine 5'-Triphosphate Solution (ATP)  
(Prepare 15 ml in Reagent A using Adenosine 5'-Triphosphate, Disodium Salt.)

C.  17 mM Phospho(enol)pyruvate Solution (PEP)  
(Prepare 15 ml in Reagent A using Phospho(enol)pyruvate, Tri(cyclohexylammonium) Salt.)

D.  555 mM Potassium Chloride Solution (KCl)  
(Prepare 15 ml in Reagent A using Potassium Chloride)
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**REAGENTS:** (continued)

E. 89 mM Magnesium Sulfate Solution (MgSO₄)  
(Prepare 15 ml in Reagent A using Magnesium Sulfate,  
Heptahydrate.)

F. 85 mM Potassium Bicarbonate Solution (KHCO₃)  
(Prepare 15 ml in Reagent A using Potassium  
Bicarbonate.)

G. 4.5 mM β-Nicotinamide Adenine Dinucleotide, Reduced  
Form Solution (β-NADH)  
(Prepare 1 ml in deionized water using β-Nicotinamide  
Adenine Dinucleotide, Reduced Form, Disodium Salt)

H. 300 mM Urea Solution (Urea)  
(Prepare 10 ml in deionized water using Urea.)

I. PK/LD Enzymes Suspension¹ (PK/LDH)  
(Use PK/LDH Enzymes Suspension.)

J. 50 mM Tris HCl, pH 8.0 at 30°C (Enzyme Diluent)  
(Prepare 50 ml in deionized water using Trizma Base.  
Adjust to pH 8.0 at 30°C with  
1 M HCl.)

K. Urease (ATP-Hydrolyzing) Enzyme Solution (UA)  
(Immediately before use, prepare a solution containing  
0.2 - 0.4 unit/ml of Urease (ATP-Hydrolyzing) in cold  
Reagent J.)

**PROCEDURE:**

Prepare a reaction cocktail by pipetting (in milliliters)  
the following reagents into a suitable containers:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Buffer)</td>
<td>49.50</td>
</tr>
<tr>
<td>B (ATP)</td>
<td>10.00</td>
</tr>
<tr>
<td>C (PEP)</td>
<td>10.00</td>
</tr>
<tr>
<td>D (KCl)</td>
<td>10.00</td>
</tr>
<tr>
<td>E (MgSO₄)</td>
<td>10.00</td>
</tr>
<tr>
<td>F (KHCO₃)</td>
<td>10.00</td>
</tr>
<tr>
<td>I (PK/LDH)</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Mix by swirling.
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PROCEDURE:  (continued)

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Cocktail</td>
<td>2.70</td>
<td>2.70</td>
</tr>
<tr>
<td>Reagent G (β-NADH)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent H (Urea)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 30°C. Monitor the A$_{340}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent K (UA)</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent J (Enzyme Diluent)</td>
<td>------</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the decrease in A$_{340}$ for approximately 5 minutes. Obtain the 6 A$_{340}$/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

\[
\text{Units/mg} \text{ enzyme} = \frac{6 \text{ A}_{340}/\text{min Test} - 6 \text{ A}_{340}/\text{min Blank}}{ (6.22) (\text{mg enzyme/ml RM})} \]

6.22 = Millimolar extinction coefficient of β-NADH at 340 nm
RM = Reaction Mix

UNIT DEFINITION:

One unit will liberate 2 µmoles of NH$_3$ from 1 µmole of urea per minute at pH 8.0 at 30°C in a coupled system with PK/LDH.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 91 mM Tris, 1.0 mM ATP, 1.5 mM phospho(enol)pyruvate, 50 mM potassium chloride, 8.0 mM magnesium sulfate, 7.7 mM potassium bicarbonate, 0.15 mM β-NADH, 10 mM urea, 3.2 units pyruvate kinase, 4.5 units lactic dehydrogenase, and 0.02 - 0.04 unit urease (ATP-hydrolyzing).
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REFERENCE:


NOTES:

1. Contains not less than 700 Pyruvate Kinase units and 1000 Lactic Dehydrogenase units per ml.

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

3. Unit Definition for Pyruvate Kinase: One unit will convert 1.0 µmole of phospho(enol)pyruvate to pyruvate per minute at pH 7.6 at 37°C.

This procedure is for informational purposes.