Enzymatic Assay of GLUTAMINE SYNTHETASE
( EC 6.3.1.2)

PRINCIPLE:

Glutamine Synthetase
Glutamate + NH$_4^+$ + ATP $\rightarrow$ L-Glutamine + ADP + P$_i$

Pyruvate Kinase
ADP + Phospho(enol)pyruvate $\rightarrow$ ATP + Pyruvate

L-Lactic Dehydrogenase
Pyruvate + $\beta$-NADH $\rightarrow$ L-Lactate + $\beta$-NAD

Abbreviations used:
ATP = Adenosine 5'-Triphosphate
ADP = Adenosine 5'-Diphosphate
P$_i$ = Inorganic Phosphate
$\beta$-NADH = $\beta$-Nicotinamide Adenine Dinucleotide, Reduced Form
$\beta$-NAD = $\beta$-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: T = 37° C, pH = 7.1, A$_{340\text{nm}}$, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Imidazole HCl Buffer, pH 7.1 at 37° C
(Prepare 200 ml in deionized water using Imidazole. Adjust to pH 7.1 at 37° C with 1 M HCl.)

B. 3 M Sodium Glutamate Solution (Glu)
(Prepare 10 ml in deionized water using L-Glutamic Acid, Monosodium Salt.)

C. 250 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 5 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt. PREPARE FRESH.)
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REAGENTS: (continued)

D. 33 mM Phospho(enol)pyruvate Solution (PEP)
   (Prepare 10 ml in deionized water using Phospho(enol)pyruvate, Trisodium Salt, Hydrate. PREPARE FRESH.)

E. 900 mM Magnesium Chloride Solution (MgCl₂)
   (Prepare 10 ml in deionized water using Magnesium Chloride, Hexahydrate.)

F. 1 M Potassium Chloride Solution (KCl)
   (Prepare 5 ml in deionized water using Potassium Chloride.)

G. 1.2 M Ammonium Chloride Solution (NH₄Cl)
   (Prepare 5 ml in deionized water using Ammonium Chloride.)

H. 12.8 mM β-Nicotinamide Adenine Dinucleotide Solution, Reduced Form (β-NADH)
   (Dissolve the contents of one 10 mg vial of β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, in the appropriate volume of Reagent A. PREPARE FRESH.)

I. PK/LDH Enzymes Solution
   (Use PK/LDH Enzymes Solution in 50% Glycerol.)

J. Glutamine Synthetase Enzyme Solution
   (Immediately before use, prepare a solution containing 4 - 8 units/ml of Glutamine Synthetase in cold deionized water.)

PROCEDURE:

Prepare a Reaction Cocktail by pipetting (in milliliters) the following reagents into a suitable container:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>20.60</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>17.20</td>
</tr>
<tr>
<td>Reagent B (Glu)</td>
<td>1.80</td>
</tr>
<tr>
<td>Reagent C (ATP)</td>
<td>1.80</td>
</tr>
<tr>
<td>Reagent E (MgCl₂)</td>
<td>3.55</td>
</tr>
<tr>
<td>Reagent F (KCl)</td>
<td>0.90</td>
</tr>
<tr>
<td>Reagent G (NH₄Cl)</td>
<td>1.80</td>
</tr>
</tbody>
</table>

Mix by stirring and adjust to pH 7.1 at 37°C with 0.1 N HCl or 0.1 N NaOH, if necessary.

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

<table>
<thead>
<tr>
<th>Reagent</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 D (PEP)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent H (β-NADH)</td>
<td>0.06</td>
<td>0.06</td>
</tr>
</tbody>
</table>
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PROCEDURE: (continued)

Mix by inversion and equilibrate to 37°C. Monitor the $A_{340\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

<table>
<thead>
<tr>
<th>Reagent I (PK/LDH)</th>
<th>0.04</th>
<th>0.04</th>
</tr>
</thead>
</table>

Mix by inversion and equilibrate to 37°C. Monitor the $A_{340\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

| Deionized water | ------ | 0.10 |
| Reagent J (Enzyme Solution) | 0.10 | ------ |

Immediately mix by inversion and record the decrease in $A_{340\text{nm}}$ for approximately 10 minutes. Obtain the $\Delta A_{340\text{nm}}$/min using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(\Delta A_{340\text{nm}}/\text{min Test} - \Delta A_{340\text{nm}}/\text{min Blank})(3)(15)}{(6.22)(0.1)}
\]

- $3 = \text{Total volume (in milliliters) of assay}$
- $15 = \text{Conversion factor to 15 minutes (Unit Definition)}$
- $6.22 = \text{Millimolar extinction coefficient of } \beta\text{-NADH at 340 nm}$
- $0.1 = \text{Volume (in milliliter) of enzyme used}$

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

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

UNIT DEFINITION:

One unit will convert 1.0 µmole of L-glutamate to L-glutamine in 15 minutes at pH 7.1 at 37°C.
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FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 34.1 mM imidazole, 102 mM sodium glutamate, 8.5 mM adenosine 5’-triphosphate, 1.1 mM phosphoenolpyruvate, 60 mM magnesium chloride, 18.9 mM potassium chloride, 45 mM ammonium chloride, 0.25 mM β-nicotinamide adenine dinucleotide, 28 units pyruvate kinase, 40 units L-lactic dehydrogenase and 0.4 - 0.8 unit glutamine synthetase.

REFERENCES:


NOTES:

1. Contains approximately 700 units/ml pyruvate kinase and 1,000 units/ml lactic dehydrogenase.

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

4. This assay is a modification of the assay procedure which is described in the cited reference.

This procedure is for informational purposes.