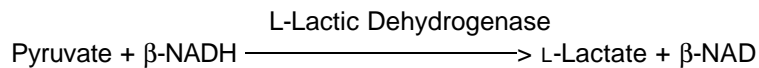
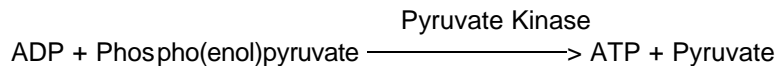
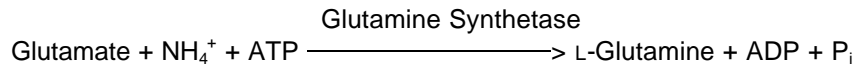


Enzymatic Assay of GLUTAMINE SYNTHETASE (EC 6.3.1.2)

PRINCIPLE:



Abbreviations used:

ATP = Adenosine 5'-Triphosphate

ADP = Adenosine 5'-Diphosphate

P_i = Inorganic Phosphate

β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form

β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: T = 37°C, pH = 7.1, A_{340nm}, 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, Sigma Prod. No. I-0250. 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, Sigma Prod. No. G-1626.)
- C. 250 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 5 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394. **PREPARE FRESH.**)

**Enzymatic Assay of GLUTAMINE SYNTHETASE
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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, Sigma Prod. No. P-7002. **PREPARE FRESH.**)
- E. 900 mM Magnesium Chloride Solution (MgCl₂)
(Prepare 10 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)
- F. 1 M Potassium Chloride Solution (KCl)
(Prepare 5 ml in deionized water using Potassium Chloride, Sigma Prod. No. P-4504.)
- G. 1.2 M Ammonium Chloride Solution (NH₄Cl)
(Prepare 5 ml in deionized water using Ammonium Chloride, Sigma Prod. No. A-4514.)
- 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, Sigma Stock No. 340-110 in the appropriate volume of Reagent A. **PREPARE FRESH.**)
- I. PK/LDH Enzymes Solution¹ (PK/LDH)
(Use PK/LDH Enzymes Solution in 50% Glycerol, Sigma Prod. No. P-0294.)
- 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:

Deionized Water	20.60
Reagent A (Buffer)	17.20
Reagent B (Glu)	1.80
Reagent C (ATP)	1.80
Reagent E (MgCl ₂)	3.55
Reagent F (KCl)	0.90
Reagent G (NH ₄ Cl)	1.80

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:

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	2.70	2.70
Reagent D (PEP)	0.10	0.10
Reagent H (β-NADH)	0.06	0.06

**Enzymatic Assay of GLUTAMINE SYNTHETASE
(EC 6.3.1.2)**

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:

Reagent I (PK/LDH)	0.04	0.04
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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 $\Delta A_{340\text{nm}}/\text{min}$ 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 = Total volume (in milliliters) of assay
- 15 = Conversion factor to 15 minutes (Unit Definition)
- 6.22 = Millimolar extinction coefficient of β -NADH at 340 nm
- 0.1 = 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.

Enzymatic Assay of GLUTAMINE SYNTHETASE (EC 6.3.1.2)

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:

Kingdon, H.S., Hubbard, J.S., and Stadtman, E.R. (1968) *Biochemistry* **7**, 2136-2142.

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 μ mole 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 μ mole 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.
5. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.