Enzymatic Assay of GUANYLATE KINASE (EC 2.7.4.8)

PRINCIPLE:

GMP + ATP $\xrightarrow{\text{Guanylate Kinase}}$ GDP + ADP

ADP + PEP $\xrightarrow{\text{Pyruvate Kinase}}$ ATP + Pyruvate

GDP + PEP $\xrightarrow{\text{Pyruvate Kinase}}$ GTP + Pyruvate

2 Pyruvate + 2 ß-NADH $\xrightarrow{\text{Lactic Dehydrogenase}}$ 2 Lactate + 2 ß-NAD

Abbreviations used:
GMP = Guanosine 5'-Monophosphate
ATP = Adenosine 5'-Triphosphate
GDP = Guanosine 5'-Diphosphate
ADP = Adenosine 5'-Diphosphate
PEP = Phospho(enol)phosphate
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: T = 30°C, pH = 7.5, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 200 mM Tris HCl Buffer, pH 7.5 at 30°C
(Prepare 50 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.5 at 30°C with 1 M HCl.)

B. 1 M Potassium Chloride Solution (KCl)
(Prepare 10 ml in deionized water using Potassium Chloride, Sigma Prod. No. P-4504.)

C. 60 mM Magnesium Sulfate Solution ($MgSO_4$)
(Prepare 20 ml in deionized water using Magnesium Sulfate, Heptahydrate, Sigma Prod. No. M-1880.)

D. 40 mM Phospho(enol)pyruvate Solution (PEP)
(Prepare 50 ml in deionized water using Phospho(enol)Pyruvate, Trisodium Salt, Hydrate, Sigma Prod. No. P-7002. **PREPARE FRESH**.)

Enzymatic Assay of GUANYLATE KINASE
(EC 2.7.4.8)

REAGENTS: (continued)

E. 100 mM Ethylenediaminetetraacetic Acid Solution (EDTA)
(Prepare 10 ml in deionized water using Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Hydrate, Sigma Stock No. ED4SS.)

F. 3.8 mM ß-Nicotinamide Adenine Dinucleotide, Reduced Form (ß-NADH)
(Prepare 2 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Dipotassium Salt, Sigma Prod. No. N-4505. **PREPARE FRESH.**)

G. 30 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 2 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394. **PREPARE FRESH.**)

H. PK/LDH Enzymes Suspension[1] (PK/LDH)
(Use PK/LDH Enzymes, Sigma Stock No. 40-7.)

I. 100 mM Guanosine 5'-Monophosphate Solution (GMP)
(Prepare 5 ml in deionized water using Guanosine 5'-Monophosphate, Disodium Salt, Sigma Prod. No. G-8377. **PREPARE FRESH.**)

J. Guanylate Kinase Enzyme Solution
(Immediately before use, prepare a solution containing 1.5 - 3.0 units/ml of Guanylate Kinase in cold deionized water.)

PROCEDURE:

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

Deionized Water	12.50
Reagent A (Buffer)	10.20
Reagent B (KCl)	1.50
Reagent C ($MgSO_4$)	3.00
Reagent D (PEP)	0.45
Reagent E (EDTA)	0.15
Reagent F (ß-NADH)	1.00

Mix by swirling and adjust to pH 7.5 at 30°C with 100 mM HCl or 100 mM NaOH, if necessary.

Enzymatic Assay of GUANYLATE KINASE (EC 2.7.4.8)

PROCEDURE: (continued)

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

	Test	Blank
Deionized Water	------	0.008
Reaction Cocktail	2.70	2.70
Reagent G (ATP)	0.10	0.10
Reagent H (PK/LDH)	0.05	0.05
Reagent J (Enzyme Solution)	0.008	---

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

Reagent I (GMP)	0.10	0.10

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

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(2.958)(df)}{(2)\,(6.22)\,(0.008)}$$

2.958 = Total volume (in milliliters) of enzyme assay
df = Dilution factor
2 = 2 moles of ß-NAD produced per mole of GMP utilized
6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
0.008 = Volume (in milliliters) 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 each of GMP and ATP to GDP

and ADP per minute at pH 7.5 at 30°C.

Enzymatic Assay of GUANYLATE KINASE (EC 2.7.4.8)

FINAL ASSAY CONCENTRATIONS:

In a 2.958 ml reaction mix, the final concentrations are 64.7 mM Tris, 47.5 mM potassium chloride, 5.7 mM magnesium sulfate, 0.57 mM phospho(enol)pyruvate, 0.48 mM ethylenediaminetetraacetic acid, 0.12 mM ß-nicotinamide adenine dinucleotide, reduced form, 1.0 mM adenosine 5'-triphosphate, 35 units pyruvate kinase, 50 units lactic dehydrogenase, 3.4 mM guanosine 5'-monophosphate and 0.012 - 0.024 unit guanylate kinase.

REFERENCES:

Hall, S.W. and Kühn, H. (1986) *Eur. J. Biochem.* **161**, 551-556.

NOTES:

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

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 based on the cited reference.

5. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.