Enzymatic Assay of HYPOXANTHINE-GUANINE PHOSPHORIBOSYL TRANSFERASE (EC 2.4.2.8)

PRINCIPLE:

$$PRPP + Guanine \xrightarrow[Mg^{++}]{HGPRT} GMP + Pyrophosphate$$

$$GMP + ATP \xrightarrow{GK} GDP + ADP$$

$$ADP + PEP \xrightarrow{PK} ATP + Pyruvate$$

$$GDP + PEP \xrightarrow{PK} GTP + Pyruvate$$

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

Abbreviations used:
PRPP = 5-Phosphorylribose 1-Pyrophosphate
HGPRT = Hypoxanthine-Guanine Phosphoribosyl Transferase
GMP = Guanosine 5'-Monophosphate
ATP = Adenosine 5'-Triphosphate
GK = Guanylate Kinase
GDP = Guanosine 5'-Diphosphate
ADP = Adenosine 5'-Diphosphate
PEP = Phospho(enol)pyruvate
PK = Pyruvate Kinase
GTP = Guanosine 5'-Triphosphate
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
LDH = Lactic Dehydrogenase
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: T = 37°C, pH = 7.5, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 71 mM Tris HCl Buffer, pH 7.5 at 37°C
(Prepare 50 ml in deionized water using Trizma Hydrochloride, Sigma Prod. No. T-3253. Adjust to pH 7.5 at 37°C with 1 M NaOH.)

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REAGENTS: (continued)

B. 1.2 M Magnesium Sulfate Solution ($MgSO_4$)
(Prepare 5 ml in deionized water using Magnesium Sulfate, Heptahydrate, Sigma Prod. No. M-1880.)

C. 3.9 M Potassium Chloride Solution (KCl)
(Prepare 5 ml in deionized water using Potassium Chloride, Sigma Prod. No. P-4504.)

D. 42 mM 5-Phosphorylribose 1-Pyrophosphate Solution (PRPP)
(Prepare 2 ml in deionized water using 5-Phosphorylribose 1-Pyrophosphate, Sodium Salt, Sigma Prod. No. P-8296.)

E. 12 mM Guanine Solution (Guanine)
(Prepare 2 ml in deionized water using Guanine, Sigma Prod. No. G-0381.)

F. 18 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 2 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394.)

G. 30 mM Phospho(enol)pyruvate Solution (PEP)
(Prepare 2 ml in deionized water using Phospho(enol)pyruvate, Trisodium Salt, Hydrate, Sigma Prod. No. P-7002.)

H. 4.8 mM ß-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (ß-NADH)
(Prepare 2 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129.)

I. Guanylate Kinase Enzyme Solution[1] (GK)
(Use Guanylate Kinase, Sigma Prod. No. G-9385.)

J. PK/LDH Enzyme Suspension[2] (PK/LDH)
(Use PK/LDH Enzymes Suspension, Sigma Stock No. 40-7.)

K. Hypoxanthine-Guanine Phosphoribosyl Transferase Enzyme Solution (HGPRT)
(Immediately before use, prepare a solution containing 200 - 250 unit/ml of Hypoxanthine-Guanine Phosphoribosyl Transferase in cold deionized water.)

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PROCEDURE:

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

Reagent A (Buffer)	21.00
Reagent B ($MgSO_4$)	1.00
Reagent C (KCl)	1.00
Reagent E (Guanine)	0.50
Reagent F (ATP)	1.00
Reagent G (PEP)	1.00
Reagent H (ß-NADH)	1.00

Mix by swirling and adjust to pH 7.5 at 37°C with 100 mM HCl or 100 mM NaOH, if necessary.

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

	Test	Blank
Reaction Cocktail	2.65	2.65
Reagent I (GK)	0.10	0.10
Reagent J (PK/LDH)	0.05	0.05
Reagent K (HGPRT)	0.10	0.10

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

Reagent D (PRPP)	0.10	------
Deionized Water	------	0.10

Immediately mix by inversion and record the decrease in A_{340nm} for approximately 5 minutes. Obtain the ΔA_{340nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{340nm}/\text{min Test} - A_{340nm}/\text{min Blank})(3)(df)}{(2)(0.00622)(0.1)}$$

3 = Total volume (in milliliters) of assay
df = Dilution factor
2 = 2 moles of ß-NAD produced per mole of GMP produced
0.00622 = µmolar extinction coefficient of ß-NADH at 340 nm
0.1 = Volume (in milliliter) of enzyme used

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CALCULATIONS:

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

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

UNIT DEFINITION:

One unit will catalyze the formation of 1 nmole of guanosine 5'-monophosphate (GMP) per minute from guanine and phosphoribosyl pyrophosphate at pH 7.5 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 49.7 mM Tris, 40 mM magnesium sulfate, 130 mM potassium chloride, 0.2 mM guanine, 0.6 mM adenosine 5'-triphosphate,
1 mM phospho(enol)pyruvate, 0.16 mM ß-nicotinamide adenine dinucleotide, reduced form, 1.4 mM phosphoribosyl pyrophosphate, 35 units pyruvate kinase, 50 units L-lactic dehydrogenase, 1 - 2 units guanylate kinase, and 20 - 25 units hypoxanthine-guanine phosphoribosyl transferase.

REFERENCES:

Giacomello, A. and Salerno, C. (1977) *Analytical Biochemistry* **79**, 263-267

NOTES:

1. The activity of Guanylate Kinase, Sigma Prod. No. G-9385, is approximately 10 - 20 units per ml.

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

3. Guanylate Kinase 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.

4. 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.

NOTES: (continued)

5. 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.

6. This assay is based on the cited reference.

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