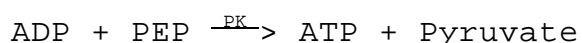
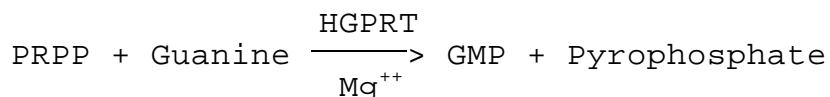


**Enzymatic Assay of HYPOXANTHINE-GUANINE  
PHOSPHORIBOSYL TRANSFERASE  
(EC 2.4.2.8)**

**PRINCIPLE:**



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

$\beta$ -NADH =  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form

LDH = Lactic Dehydrogenase

$\beta$ -NAD =  $\beta$ -Nicotinamide Adenine Dinucleotide, Oxidized Form

**CONDITIONS:** T = 37°C, pH = 7.5, A<sub>340nm</sub>, 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 ( $\text{MgSO}_4$ )  
(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  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form Solution ( $\beta$ -NADH)  
(Prepare 2 ml in deionized water using  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129.)
- I. Guanylate Kinase Enzyme Solution<sup>1</sup> (GK)  
(Use Guanylate Kinase, Sigma Prod. No. G-9385.)
- J. PK/LDH Enzyme Suspension<sup>2</sup> (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 <sub>4</sub> )	1.00
Reagent C (KCl)	1.00
Reagent E (Guanine)	0.50
Reagent F (ATP)	1.00
Reagent G (PEP)	1.00
Reagent H (β-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:

	<u>Test</u>	<u>Blank</u>
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<sub>340nm</sub> 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<sub>340nm</sub> for approximately 5 minutes. Obtain the r A<sub>340nm</sub>/minute using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

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

3 = Total volume (in milliliters) of assay

df = Dilution factor

2 = 2 moles of β-NAD produced per mole of GMP produced

0.00622 = μmolar extinction coefficient of β-NADH at 340 nm

0.1 = Volume (in milliliter) of enzyme used

**Enzymatic Assay of HYPOXANTHINE-GUANINE  
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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  $\beta$ -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  $\mu$ mole 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  $\mu$ mole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.

**Enzymatic Assay of HYPOXANTHINE-GUANINE  
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**NOTES:** (continued)

5. Pyruvate Kinase Unit Definition: One unit will convert 1.0  $\mu$ mole 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.

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