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

**PRINCIPLE:**

\[
\text{HGPRT} \\
\text{PRPP + Guanine} \xrightarrow{\text{Mg}^{++}} \text{GMP + Pyrophosphate} \\
\text{GMP + ATP} \xrightarrow{\text{GK}} \text{GDP + ADP} \\
\text{ADP + PEP} \xrightarrow{\text{PK}} \text{ATP + Pyruvate} \\
\text{GDP + PEP} \xrightarrow{\text{PK}} \text{GTP + Pyruvate} \\
2 \text{Pyruvate} + 2 \beta-\text{NADH} \xrightarrow{\text{LDH}} 2 \text{Lactate} + 2 \beta-\text{NAD}
\]

Abbreviations used:
- PRPP = 5-Phosphoribosyl 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:**  \(T = 37^\circ \text{C}, \text{pH} = 7.5, A_{340nm}\), 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 (MgSO₄)  
(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-Phosphorylribosyl 1-Pyrophosphate Solution (PRPP)  
(Prepare 2 ml in deionized water using 5-Phosphorylribosyl 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 Solution¹ (GK)  
(Use Guanylate Kinase, Sigma Prod. No. G-9385.)

J. PK/LDH Enzyme Suspension² (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:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Buffer)</td>
<td>21.00</td>
</tr>
<tr>
<td>B (MgSO₄)</td>
<td>1.00</td>
</tr>
<tr>
<td>C (KCl)</td>
<td>1.00</td>
</tr>
<tr>
<td>E (Guanine)</td>
<td>0.50</td>
</tr>
<tr>
<td>F (ATP)</td>
<td>1.00</td>
</tr>
<tr>
<td>G (PEP)</td>
<td>1.00</td>
</tr>
<tr>
<td>H (β-NADH)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Cocktail</td>
<td>2.65</td>
<td>2.65</td>
</tr>
<tr>
<td>I (GK)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>J (PK/LDH)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>K (HGPRT)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 37°C. Monitor the A₃₄₀nm until constant, using a suitably thermostatted spectrophotometer. Then add:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D (PRPP)</td>
<td>0.10</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the decrease in A₃₄₀nm for approximately 5 minutes. Obtain the r A₃₄₀nm/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

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
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CALCULATIONS:

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

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

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 β-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:


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.
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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.

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