**Enzymatic Assay of RIBOKINASE**
(EC 2.7.1.15)

**PRINCIPLE:**

\[
\text{ATP} + \text{d-Ribose} \xrightarrow{\text{Ribokinase}} \text{ADP} + \text{d-Ribose 5-P}
\]

\[
\text{d-Ribose 5-P} \xrightarrow{\text{PRI}} \text{d-Ribulose 5-P}
\]

\[
\text{d-Ribulose 5-P} \xrightarrow{\text{Ru-5-P-3-Epim}} \text{d-Xylulose 5-P}
\]

\[
\text{d-Xylulose 5-P} + \text{d-Ribose 5-P} \xrightarrow{\text{TK}} \text{GAP} + \text{Sedoheptulose 7-P}
\]

\[
\text{GAP} + \beta-\text{NADH} \xrightarrow{\text{a-GDH}} \beta-\text{NAD} + \text{a-Glycerophosphate}
\]

**Abbreviations used:**

- ADP = Adenosine 5'-Phosphate
- PRI = Phosphoriboisomerase
- Ru-5-P-3-Epim = Ribulose-5-Phosphate-3-Epimerase
- TK = Transketolase
- GAP = Glyceraldehyde 3-Phosphate
- TPI = Triosephosphate Isomerase
- DHAP = Dihydroxyacetone Phosphate
- \(\beta-\text{NADH}\) = \(\beta-\text{Nicotinamide Adenine Dinucleotide, Reduced Form}\)
- \(\alpha-\text{GDH}\) = \(\alpha-\text{Glycerophosphate Dehydrogenase}\)
- \(\beta-\text{NAD}\) = \(\beta-\text{Nicotinamide Adenine Dinucleotide, Oxidized Form}\)

**CONDITIONS:**  
T = 37°C, pH = 7.7, \(A_{340nm}\), Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENT:**

A. 250 mM Glycylglycine Buffer, pH 7.7 at 37°C  
(Prepare 100 ml in deionized water using Glycylglycine. Adjust to pH 7.7 at 37°C with 1 M NaOH.)
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REAGENTS:  (continued)

B. 100 mM Ribose Solution (Ribose)  
(Prepare 1 ml in deionized water using D(-)Ribose.)

C. 2 mM Cocarboxylase Solution (Cocarboxylase)  
(Prepare 1 ml in deionized water using Cocarboxylase.)

D. 2.5 mM β-Nicotinamide Adenine Dinucleotide Solution,  
Reduced Form (β-NADH)  
(Dissolve the contents of one 5 mg vial of β-Nicotinamide Adenine Dinucleotide, Reduced Form,  
Disodium Salt, in the appropriate volume of deionized water.)

E. 300 mM Magnesium Chloride Solution (MgCl₂)  
(Prepare 1 ml in deionized water using Magnesium Chloride, Hexahydrate.)

F. 180 mM Adenosine 5'-Triphosphate Solution (ATP)  
(Prepare 1 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt.)

G. Ribulose-5-Phosphate-3-Epimerase Enzyme Solution  
(Ru-5-P-3-Epim)  
(Immediately before use, prepare a solution containing 10 units/ml of D-Ribulose 5-Phosphate-3-Epimerase,  
in cold deionized water.)

H. Transketolase Enzyme Solution (TK)  
(Immediately before use, prepare a solution containing 10 units/ml of Transketolase, in cold deionized water.)

I. a-Glycerophosphate Dehydrogenase/Triosephosphate  
Isomerase Enzyme Solution (a-GDH/TPI)  
(Immediately before use, prepare a solution containing 100 units/ml of a-Glycerophosphate Dehydrogenase-  
Triosephosphate Isomerase, in cold deionized water.  
The 100 units/ml is based on a-GDH units.)

J. Phosphoriboisomerase Enzyme Solution (PRI)  
(Immediately before use, prepare a solution containing 50 units/ml of Phosphoriboisomerase,  
in cold deionized water.)
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REAGENTS:  (continued)

K. Ribokinase Enzyme Solution (Ribokinase)  
(Immediately before use, prepare a solution containing 0.3 - 0.5 unit/ml of Ribokinase in cold Reagent A.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.25</td>
<td>2.35</td>
</tr>
<tr>
<td>Reagent B (Ribose)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (Cocarboxylase)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent D (β-NADH)</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Reagent E (MgCl₂)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent F (ATP)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent G (Ru-5-P-3-Epim)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent H (TK)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent I (a-GDH/TPI)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent J (PRI)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

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

Reagent K (Ribokinase) 0.10

Immediately mix by inversion and record the decrease in $A_{340\text{nm}}$ for approximately 15 minutes. The maximum linear rate usually occurs between 10 - 15 minutes. Obtain the $r_{A_{340\text{nm}}/\text{min}}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(r_{A_{340\text{nm}}/\text{min Test}} - r_{A_{340\text{nm}}/\text{min Blank}})(3)(df)}{(6.22)(0.1)}
\]

3 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of β-NADH at 340nm
0.1 = Volume (in milliliter) of enzyme used
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CALCULATIONS: (continued)

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

UNIT DEFINITION:

One unit will convert 1.0 µmole of \(\text{D-ribose}\) to \(\text{D-ribose 5-phosphate}\) per minute at pH 7.7 at 37°C in the presence of ATP and magnesium.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 196 mM glycylglycine, 3.3 mM \(\text{D-ribose}\), 0.03 mM cocarboxylase, 0.13 mM \(\text{B-nicotinamide adenine dinucleotide, reduced form}\), 10 mM magnesium chloride, 3 mM adenosine 5'-triphosphate, 0.5 unit ribulose-5-phosphate-3-epimerase, 0.5 unit transketolase, 5 units \(\text{a-glycerophosphate dehydrogenase}\), approximately 50 units triosephosphate isomerase, 2.5 units phosphoriboisomerase, and 0.03 - 0.05 unit ribokinase.

REFERENCE:

Agranoff, B.W. and Brady, R.O. (1956) Journal of Biological Chemistry 219, 221-229


NOTES:

1. Phosphoriboisomerase Unit Definition: One unit will convert 1.0 µmole of \(\text{D-ribose 5-phosphate}\) to \(\text{D-ribulose 5-phosphate}\) per minute at pH 7.7 at 30°C.

2. \(\text{D-Ribulose-5-Phosphate-3-Epimerase Unit Definition:}\) One unit will convert 1 µmole of \(\text{D-ribulose 5-phosphate}\) to \(\text{D-xylulose 5-phosphate}\) per minute at pH 7.7 at 25°C in a coupled system with ribose-5-phosphate, \(\text{B-NADH, transketolase, a-glycerophosphate dehydrogenase/triosephosphate isomerase and cocarboxylase}\).
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NOTES:  (continued)

3. Transketolase Unit Definition: One unit will produce 1 µmole of glyceraldehyde 3-phosphate from xylulose 5-phosphate per minute at pH 7.7 at 25°C in the presence of ribose 5-phosphate, cocarboxylase, and magnesium in a coupled system with triosephosphate isomerase and a-glycerophosphate dehydrogenase.

4. a-Glycerophosphate Dehydrogenase Unit Definition: One unit will convert 1.0 µmole of dihydroxyacetone phosphate to a-glycerophosphate per minute at pH 7.4 and 25°C.

5. Triosephosphate Isomerase Unit Definition: One unit will convert 1.0 µmole of D-glyceraldehyde 3-phosphate to dihydroxyacetone phosphate per minute at pH 7.6 and 25°C.

6. This assay is based on the cited references.