Enzymatic Assay of RIBOKINASE
(EC 2.7.1.15)

PRINCIPLE:

ATP + D-Ribose $\xrightarrow{\text{Ribokinase}}$ ADP + D-Ribose 5-P

D-Ribose 5-P $\xrightarrow{\text{PRI}}$ D-Ribulose 5-P

D-Ribulose 5-P $\xrightarrow{\text{Ru-5-P-3-Epim}}$ D-Xylulose 5-P

D-Xylulose 5-P + D-Ribose 5-P $\xrightarrow[\text{Mg++, Cocarboxylase}]{\text{TK}}$ GAP + Sedoheptulose 7-P

GAP + $\xrightarrow{\text{TPI}}$ DHAP

DHAP + ß-NADH $\xrightarrow{\text{a-GDH}}$ ß-NAD + 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
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
a-GDH = a-Glycerophosphate Dehydrogenase
ß-NAD = ß-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, Sigma Prod. No. G-1002. Adjust to pH 7.7 at 37°C with 1 M NaOH.)

REAGENTS: (continued)

B. 100 mM Ribose Solution (Ribose)
(Prepare 1 ml in deionized water using D(-)Ribose, Sigma Prod. No. R-7500.)

C. 2 mM Cocarboxylase Solution (Cocarboxylase)
(Prepare 1 ml in deionized water using Cocarboxylase, Sigma Prod. No. C-8754.)

D. 2.5 mM ß-Nicotinamide Adenine Dinucleotide Solution, Reduced Form (ß-NADH)
(Dissolve the contents of one 5 mg vial of ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-105 in the appropriate volume of deionized water.)

E. 300 mM Magnesium Chloride Solution ($MgCl_2$)
(Prepare 1 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)

F. 180 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 1 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-2383.)

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, Sigma Prod. No. R-3251, in cold deionized water.)

H. Transketolase Enzyme Solution (TK)
(Immediately before use, prepare a solution containing 10 units/ml of Transketolase, Sigma Prod. No. T-6133, 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, Sigma prod. No. G-1881, 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, Sigma Prod. No. P-1780, 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:

	Test	Blank
Reagent A (Buffer)	2.25	2.35
Reagent B (Ribose)	0.10	0.10
Reagent C (Cocarboxylase)	0.05	0.05
Reagent D (ß-NADH)	0.15	0.15
Reagent E ($MgCl_2$)	0.10	0.10
Reagent F (ATP)	0.05	0.05
Reagent G (Ru-5-P-3-Epim)	0.05	0.05
Reagent H (TK)	0.05	0.05
Reagent I (a-GDH/TPI)	0.05	0.05
Reagent J (PRI)	0.05	0.05

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

Reagent K (Ribokinase)	0.10	------

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

CALCULATIONS:

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

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

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 D-ribose to 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 D-ribose, 0.03 mM cocarboxylase, 0.13 mM ß-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 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

Racker, E. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed) 2nd ed., Volume III, 1342-1345, Academic Press, New York, NY

NOTES:

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

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

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.

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