

**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  $\xrightarrow[\text{Mg}^{++}, \text{Coccarboxylase}]{\text{TK}}$  GAP + Sedoheptulose 7-P

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

DHAP +  $\beta$ -NADH  $\xrightarrow{\text{a-GDH}}$   $\beta$ -NAD +  $\alpha$ -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

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

$\alpha$ -GDH =  $\alpha$ -Glycerophosphate Dehydrogenase

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

**CONDITIONS:** T = 37°C, pH = 7.7, A<sub>340nm</sub>, 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.)

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**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  $\beta$ -Nicotinamide Adenine Dinucleotide Solution, Reduced Form ( $\beta$ -NADH)  
(Dissolve the contents of one 5 mg vial of  $\beta$ -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.  $\alpha$ -Glycerophosphate Dehydrogenase/Triosephosphate Isomerase Enzyme Solution ( $\alpha$ -GDH/TPI)  
(Immediately before use, prepare a solution containing 100 units/ml of  $\alpha$ -Glycerophosphate Dehydrogenase-Triosephosphate Isomerase, Sigma prod. No. G-1881, in cold deionized water. The 100 units/ml is based on  $\alpha$ -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 (Coccarboxylase)	0.05	0.05
Reagent D ( $\beta$ -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 ( $\alpha$ -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	-----
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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  
 $r_{A_{340nm}/min}$  using the maximum linear rate for both the Test  
and Blank.

**CALCULATIONS:**

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

3 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of  $\beta$ -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  $\mu$ mole 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  $\beta$ -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  $\alpha$ -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  $\mu$ mole 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  $\mu$ mole 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,  $\beta$ -NADH, transketolase,  $\alpha$ -glycerophosphate dehydrogenase/triosephosphate isomerase and cocarboxylase.

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**NOTES:** (continued)

3. Transketolase Unit Definition: One unit will produce 1  $\mu$ mole 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  $\alpha$ -glycerophosphate dehydrogenase.
4.  $\alpha$ -Glycerophosphate Dehydrogenase Unit Definition: One unit will convert 1.0  $\mu$ mole of dihydroxyacetone phosphate to  $\alpha$ -glycerophosphate per minute at pH 7.4 and 25°C.
5. Triosephosphate Isomerase Unit Definition: One unit will convert 1.0  $\mu$ mole 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.

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