

**Enzymatic Assay of PHOSPHORIBOISOMERASE  
(EC 5.3.1.6)**

**PRINCIPLE:**

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 + Ribose 5-P  $\xrightarrow[\text{MG}^{++}, \text{cocarboxylase}]{\text{TK}}$  GAP + Sedoheptulose 7-P

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

DHAP +  $\beta$ -NADH + H<sup>+</sup>  $\xrightarrow{\alpha\text{-GDH}}$   $\beta$ -NAD +  $\alpha$ -Glycerophosphate

Abbreviations used:

PRI = Phosphoriboisomerase

Ru-5-P-3-Epim. = Ribulose 5-Phosphate-3-Epimerase

GAP = Glyceraldehyde 3-Phosphate

TK = Transketolase

TPI = Triosephosphate Isomerase

$\beta$ -NADH =  $\beta$ -Nicotinamide Adenine Dinucleotide, reduced form

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

DHAP = Dihydroxyacetone Phosphate

$\beta$ -NAD =  $\beta$ -Nucleotide Adenine Dinucleotide, oxidized form

**CONDITIONS:** T = 30°C, pH = 7.7, A<sub>340nm</sub>, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

- A. 250 mM Glycylglycine Buffer, pH 7.7 at 30°C  
(Prepare 100 ml in deionized water using Glycylglycine Buffer, Prod. No. G-1002. Adjust to pH 7.7 at 30°C using 5 N NaOH.)
- B. 100 mM D-Ribose 5-Phosphate Solution (R-5-P)  
(Prepare 1.0 ml in deionized water using D-Ribose 5'-Phosphate Disodium, Prod. No. R-7750.)

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**REAGENTS:** (continued)

- C. 2.0 mM Cocarboxylase Solution  
(Prepare 1.0 ml in deionized water using Cocarboxylase, Prod. No. C-8754. **PREPARE FRESH.**)
- D. 2.5 mM  $\beta$ -NADH Solution  
(Dissolve the contents of 1 vial of  $\beta$ -NADH Disodium, Stock No. 340-105, in 2.5 ml in Reagent A.)
- E. 300 mM Magnesium Chloride Solution ( $MgCl_2$ )  
(Prepare 1 ml in deionized water using Magnesium Chloride, Hexahydrate, Prod. No. M-0250.)
- F. Ribulose 5-Phosphate-3-Epimerase Enzyme Solution (Ru 5-P-3-Epim.)  
(Immediately before use, prepare a solution containing 10 units/ml of  $\beta$ -Ribulose 5-Phosphate-3-Epimerase, Prod. No. R-3251 in deionized water.)
- G. Transketolase Enzyme Solution (TK)  
(Immediately before use, prepare a solution containing 10 units/ml of Transketolase Enzyme Solution, Prod. No. T-6133, in deionized water.)
- H.  $\alpha$ -Glycerophosphate Dehydrogenase-Triosephosphate Isomerase Solution ( $\alpha$ -GDH-TPI)  
(Immediately before use, prepare a solution containing 100 units/ml of  $\alpha$ -Glycerophosphate Dehydrogenase-Triosephosphate Isomerase, Prod. No. G-1881, in deionized water. The 100 units/ml is based on  $\alpha$ -GDH units.)
- I. Phosphoriboisomerase Enzyme Solution (PRI)  
(Immediately before use, prepare a solution containing 0.25 to 1.0 of Phosphoriboisomerase using cold Reagent A.)

**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.35	2.35
Reagent B (R-5-P)	0.10	0.10
Reagent C (Cocarboxylase)	0.05	0.05

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**PROCEDURE:** (continued)

	Test	Blank
Reagent D ( $\beta$ -NADH)	0.15	0.15
Reagent E ( $MgCl_2$ )	0.10	0.10
Reagent F (Ru-5-P-3-Epim)	0.05	0.05
Reagent G (TK)	0.05	0.05
Reagent H ( $\alpha$ -GDH-TPI)	0.05	0.05

Mix by inversion and equilibrate to 30°C. Monitor the  $A_{340nm}$  until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent I	0.10	-----
Reagent A	-----	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  $r A_{340nm}/\text{minute}$  using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/mg enzyme} = \frac{r A_{340nm}/\text{min Test} - r A_{340}/\text{min Blank}}{(6.22) (\text{mg enzyme/ml RM})}$$

6.22 = Millimolar extinction coefficient of  $\beta$ -NAD at 340 nm  
 RM = Reaction Mix

**UNIT DEFINITION:**

One unit will convert 1.0  $\mu\text{mole}$  of D-ribose 5-phosphate to D-ribulose 5-phosphate per minute at pH 7.7 at 30°C.

**FINAL ASSAY CONCENTRATION:**

In a 3.0 ml reaction mix, the final concentrations are 217 mM glycylglycine, 3.33 mM D-ribose 5-phosphate, 0.033 mM cocarboxylase, 0.125 mM  $\beta$ -NADH, 10.0 mM magnesium chloride, 0.5 units ribulose 5-phosphate-3-epimerase, 0.5 units transketolase, 5.0 units  $\alpha$ -glycerophosphate dehydrogenase, approximately 50.0 units triosephosphate isomerase, and 0.025 to 0.1 units phosphoriboisomerase.

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**NOTES:**

1. 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.
2. Transketolase unit definition: One unit will produce 1  $\mu$ mole of glyceraldehyde-3-phosphate from xylulose-5-phosphate per minute of 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.
3.  $\alpha$ -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.
4. 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.
5. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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