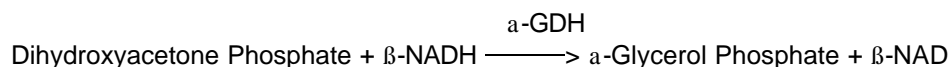
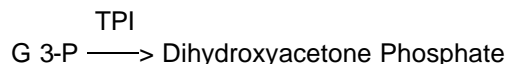
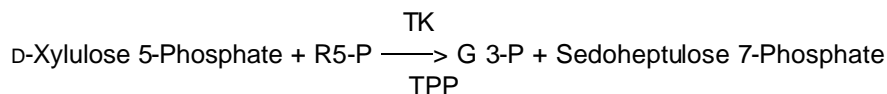
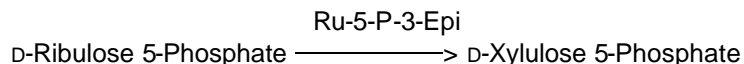


**Enzymatic Assay of D-RIBULOSE 5-PHOSPHATE 3-EPIMERASE  
(EC 5.1.3.1)**

**PRINCIPLE:**



Abbreviations used:

RU-5-P-3-Epi = D-Ribulose 5-Phosphate 3-Epimerase

TK = Transketolase

TPP = Thiamine Pyrophosphate

R5-P = D-Ribose 5-phosphate

G 3-P = Glyceraldehyde 3-Phosphate

TPI = Triosephosphate Isomerase

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

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

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

**CONDITIONS:** T = 25°C, pH = 7.7,  $A_{340\text{nm}}$ , Light path = 1 cm

**METHODS:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

- A. 250 mM Glycylglycine Buffer, pH 7.7 at 25°C  
(Prepare 100 ml in deionized water using Glycylglycine, Free Base, Sigma Prod. No. G-1002.  
Adjust to pH 7.7 at 25°C with 1 M NaOH.)
- B. 100 mM D-Ribulose 5-Phosphate Solution (RU 5-P)  
(Prepare 1 ml in deionized water using D-Ribulose 5-Phosphate, Sodium Salt, Sigma  
Prod. No. R-9875, in deionized water.)

**Enzymatic Assay of D-RIBULOSE 5-PHOSPHATE 3-EPIMERASE  
(EC 5.1.3.1)**

**REAGENTS:** (continued)

- C. 100 mM D Ribose 5-Phosphate Solution (R 5-P)  
(Prepare 1 ml in deionized water using D-Ribose 5-Phosphate, Disodium Salt, Sigma Prod. No. R-7750.)
  
- D. 0.10% (w/v) Cocarboxylase/Thiamine Pyrophosphate Solution (TPP)  
(Prepare 1 ml in cold deionized water using Cocarboxylase, Sigma Prod. No. C-8754.  
**PREPARE FRESH.**)
  
- E. 300 mM Magnesium Chloride Solution (MgCl<sub>2</sub>)  
(Prepare 5 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)
  
- F. 2.6 mM β-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β-NADH)  
(Dissolve the contents of one 5 mg vial of β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-105, in the appropriate volume of Reagent A.  
**PREPARE FRESH.**)
  
- G. α-Glycerophosphate Dehydrogenase/Triosephosphate Isomerase Enzyme Solution (α-GDH/TPI)  
(Immediately before use, prepare a solution containing 10 α-GDH units/ml of α-Glycerophosphate Dehydrogenase/ Triosephosphate Isomerase<sup>1</sup>, Sigma Prod. No. G-6755, 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. D-Ribulose 5-Phosphate 3-Epimerase Enzyme Solution (RU-5-P-3-EPI)  
(Immediately before use, prepare a solution containing 0.25-0.50 unit/ml of D-Ribulose 5-Phosphate 3-Epimerase in cold deionized water.)



## Enzymatic Assay of D-RIBULOSE 5-PHOSPHATE 3-EPIMERASE (EC 5.1.3.1)

### 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.

### FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 58 mM glycylglycine, 1.7 mM ribulose 5-phosphate, 1.7 mM ribose 5-phosphate, 0.002% (w/v) cocarboxylase, 15 mM magnesium chloride, 0.13 mM  $\beta$ -nicotinamide adenine dinucleotide, reduced form, 0.5 unit  $\alpha$ -glycerophosphate dehydrogenase, 5 units triosephosphate isomerase, 0.5 unit transketolase, and 0.025-0.05 unit D-ribulose 5-phosphate 3-epimerase.

### REFERENCE:

Wood, T. (1970) *Analytical Biochemistry* **33**, 297-306

### NOTES:

1. This product contains approximately a 10:1 ratio of triosephosphate isomerase activity to  $\alpha$ -glycerophosphate dehydrogenase activity.
2. There may be a sudden decrease in  $\beta$ -NADH within the first two minutes. This decrease may be approximately 0.1 - 0.2 per minute. Use the linear portion of the curve, after the sudden decrease, to obtain the maximum linear rate.
3. 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 at 25°C.
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 at 25°C.
5. Transketolase Unit Definition: One unit will produce 1.0  $\mu$ 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, thiamine pyrophosphate and  $Mg^{++}$  using a coupled system with  $\alpha$ -GDH/TPI.
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.**