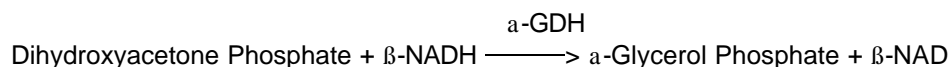
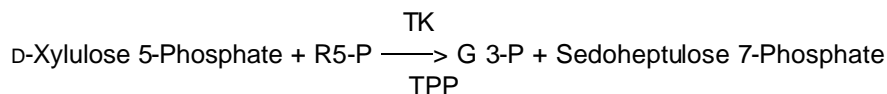
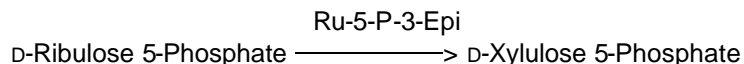


**Enzymatic Assay of D-RIBULOSE 5-PHOSPHATE 3-EPIMERASE
(EC 5.1.3.1)**

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

α -GDH = α -Glycerophosphate Dehydrogenase

β -NADH = β -Nicotinamide Adenine Dinucleotide, Reduced Form

β -NAD = β -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 (MgCl₂)
(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 Isomerase¹, 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)**

PROCEDURE:

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

		<u>Test</u>	<u>Blank</u>
Deionized Water		1.80	1.90
Reagent A (Buffer)		0.55	0.55
Reagent B (Ru5-P)	0.05	0.05	
Reagent C (R 5-P)		0.05	0.05
Reagent D (TPP)		0.05	0.05
Reagent E (MgCl ₂)		0.15	0.15
Reagent F (β-NADH)		0.15	0.15
Reagent G (α-GDH/TPI)		0.05	0.05
Reagent H (TK)		0.05	0.05

Mix by inversion and equilibrate to 25°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent I (Ru-5-P-3-Epi)		0.10	-----
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Immediately mix by inversion and record the decrease in A_{340nm} for approximately 5 minutes.² Obtain the r A_{340nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

3 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of β-NADH at 340 nm

0.1 = Volume (in milliliter) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

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

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

UNIT DEFINITION:

One unit will convert 1 μ 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, β -NADH, transketolase, α -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 β -nicotinamide adenine dinucleotide, reduced form, 0.5 unit α -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 α -glycerophosphate dehydrogenase activity.
2. There may be a sudden decrease in β -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 μ mole of D-glyceraldehyde 3-phosphate to dihydroxyacetone phosphate per minute at pH 7.6 at 25°C.
4. α -Glycerophosphate Dehydrogenase Unit Definition: One unit will convert 1.0 μ mole of dihydroxyacetone phosphate to α -glycerophosphate per minute at pH 7.4 at 25°C.
5. Transketolase Unit Definition: One unit will produce 1.0 μ 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 α -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.