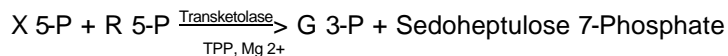


**Enzymatic Assay of TRANSKETOLASE
(EC 2.2.1.1)**

PRINCIPLE:



Abbreviations used:

X 5-P = Xylulose 5-Phosphate

R 5-P = Ribose 5-Phosphate

TPP = Thiamine Pyrophosphate

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 50 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 Xylulose 5-Phosphate Solution (X 5-P)
(Prepare 1 ml in deionized water using D-Xylulose 5-Phosphate, Sodium Salt, Sigma
Prod. No. X-3750.)
- C. 50 mM 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.)

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REAGENTS: (continued)

- D. 0.10% (w/v) Cocarboxylase (Thiamine Pyrophosphate) Solution
(Prepare 1 ml in cold deionized water using Cocarboxylase, Sigma Prod. No. C-8754.
PREPARE FRESH.)
- E. 4.3 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, or the appropriate weight of Sigma Prod. No. N-8129. **PREPARE FRESH.**)
- F. 300 mM Magnesium Chloride Solution ($MgCl_2$)
(Prepare 1 ml in deionized water using Magnesium Chloride Sterile Filtered Solution, Sigma Prod. No. M-1028.)
- G. α -Glycerophosphate Dehydrogenase/Triosephosphate Isomerase Enzyme Solution
- H. (α -GDH/TPI)
(Immediately before use, prepare a solution containing 2000 TPI units/ml of α -Glycerophosphate Dehydrogenase/ Triosephosphate Isomerase, Sigma Prod. No. G-6755, in cold deionized water.)
- H. Transketolase Enzyme Solution
(Immediately before use, prepare a solution containing 5.0 units/ml of Transketolase in cold Reagent A.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.48	2.49
Reagent B (X 5-P)	0.10	0.10
Reagent C (R 5-P)	0.10	0.10
Reagent D (Cocarboxylase)	0.05	0.05
Reagent E (β -NADH)	0.10	0.10
Reagent F ($MgCl_2$)	0.15	0.15
Reagent G (α -GDH/TPI)	0.01	0.01

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PROCEDURE: (continued)

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

Reagent H (Enzyme Solution)	0.01	-----
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Immediately mix by inversion and record the decrease in $A_{340\text{nm}}$ for approximately 10 minutes. Obtain the $r A_{340\text{nm}}$ /minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{340\text{nm}}/\text{min Test} - r A_{340\text{nm}}/\text{min Blank})(3)(\text{df})}{(6.22) (0.01)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

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

0.01 = Volume (in milliliters) of enzyme used

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

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

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.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 216 mM glycylglycine, 3.3 mM xylulose 5-phosphate, 1.7 mM ribose 5-phosphate, 0.002% (w/v) cocarboxylase, 0.14 mM β -nicotinamide adenine dinucleotide, reduced form, 15 mM magnesium chloride, 20 units α -glycerophosphate dehydrogenase/triosephosphate isomerase (based on triosephosphate isomerase units) and 0.05 unit transketolase.

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REFERENCE:

de la Haba, G., Leder, I.G., and Racker, E. (1955) *Journal of Biological Chemistry* **214**, 409-426

NOTES:

1. This assay is based on the cited reference.
2. 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.
3. a-Glycerophosphate Dehydrogenase Unit Definition: One unit will convert 1.0 μ mole of dihydroxyacetone phosphate to a-glycerophosphate per minute at pH 7.4 at 25°C.
4. 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.