Enzymatic Assay of TRANSALDOLASE
(EC 2.2.1.2)

PRINCIPLE:

$_D$-Fructose 6-Phosphate + $_D$-Erythrose 4-Phosphate $\xrightarrow{TA}$ S-7-P + GAP

GAP $\xrightarrow{TPI}$ Dihydroxyacetone Phosphate

DHAP + ß-NADH $\xrightarrow{a\text{-}GDH}$ a-Glycerophosphate + ß-NAD

Abbreviations used:
TA = Transaldolase
S-7-P = Sedoheptulose 7-Phosphate
GAP = Glyceraldehyde 3-Phosphate
TPI = Triosephosphate Isomerase
DHAP = Dihydroxyacetone Phosphate
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
a-GDH = = a-Glycerophosphate Dehydrogenase
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form

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

METHOD: 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 $_D$-Erythrose 4-Phosphate Solution (E-4-P)
(Prepare 1 ml in deionized water using $_D$-Erythrose 4-Phosphate, Sodium Salt, Sigma Prod. No. E-0377.)

C. 200 mM $_D$-Fructose 6-Phosphate Solution (F-6-P)
(Prepare 2 ml in deionized water using $_D$-Fructose 6-Phosphate, Disodium Salt, Sigma Prod. No. F-3627.)

D. 300 mM Magnesium Chloride Solution ($MgCl_2$)
(Prepare 10 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)

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REAGENTS:

E. 2.6 mM ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Solution (ß-NADH)
(Dissolve the contents of one 5 mg vial of ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt,
Sigma Stock No. 340-105, in the appropriate volume of Reagent A.)

F. a-Glycerophosphate Dehydrogenase/Triosephosphate Isomerase Enzyme Solution (a-GDH/TPI)
(Immediately before use, prepare a solution containing 0.1 mg/ml of a-Glycerophosphate Dehydrogenase/ Triosephosphate Isomerase, Sigma Prod. No. G-1881 in cold Reagent A.)

G. Transaldolase Enzyme Solution (TA)
(Immediately before use, prepare a solution containing 0.25 - 0.50 unit/ml of Transaldolase in cold deionized water.)

PROCEDURE:

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

	Test	Blank
Deionized Water	1.80	1.80
Reagent A (Buffer)	0.55	0.55
Reagent B (E-4-P)	0.05	0.05
Reagent C (F-6-P)	0.10	0.10
Reagent E (ß-NADH)	0.15	0.15
Reagent D ($MgCl_2$)	0.15	0.15
Reagent F (a-GDH/TPI)	0.10	0.10

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

Reagent G (TA)	0.10	---
Deionized Water	---	0.10

Immediately mix by inversion and record the decrease in A_{340nm} for approximately 5 minutes. Obtain the ΔA_{340nm}/minute using the maximum linear rate for both the Test and Blank.

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CALCULATIONS:

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

3 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
0.1 = Volume (in milliliter) of transaldolase 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 µmole of D-glyceraldehyde 3-phosphate from D-fructose 6-phosphate per minute in the presence of D-erythrose 4-phosphate, at pH 7.7 at 25°C in a coupled system with a-GDH/TPI and ß-NADH.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 67 mM glycylglycine, 2 mM D-erythrose 4-phosphate, 6.7 mM D-fructose 6-phosphate, 15 mM magnesium chloride, 0.13 mM ß-nicotinamide adenine dinucleotide, 0.01 mg of a-glycerophosphate dehydrogenase/triosephosphate isomerase, 0.025 - 0.050 units transaldolase.

REFERENCE:

Bergmeyer, H.U., Gawehn, K., and Grassl, M. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) Volume I, 513-514, Academic Press, Inc., New York, NY

NOTES:

1. This assay is based on the cited reference.

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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