Enzymatic Assay of TRANSALDOLASE
(EC 2.2.1.2)

PRINCIPLE:

\[ \text{D-Fructose 6-Phosphate + D-Erythrose 4-Phosphate} \xrightarrow{\text{TA}} \text{S-7-P + GAP} \]

\[ \text{GAP} \xrightarrow{\text{TPI}} \text{Dihydroxyacetone Phosphate} \]

\[ \text{DHAP + } \beta-\text{NADH} \xrightarrow{\text{a-GDH}} \alpha-\text{Glycerophosphate + } \beta-\text{NAD} \]

Abbreviations used:

TA = Transaldolase
S-7-P = Sedoheptulose 7-Phosphate
GAP = Glyceraldehyde 3-Phosphate
TPI = Triosephosphate Isomerase
DHAP = Dihydroxyacetone Phosphate
\( \beta-\text{NADH} = \beta-\text{Nicotinamide Adenine Dinucleotide, Reduced Form} \)
\( \alpha-\text{GDH} = = \alpha-\text{Glycerophosphate Dehydrogenase} \)
\( \beta-\text{NAD} = \beta-\text{Nicotinamide Adenine Dinucleotide, Oxidized Form} \)

CONDITIONS: \( T = 25^\circ \text{C}, \, \text{pH} \, 7.7, \, A_{340\text{nm}}, \, \text{Light path} = 1 \, \text{cm} \)

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 (MgCl₂)
   (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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>1.80</td>
<td>1.80</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.55</td>
<td>0.55</td>
</tr>
<tr>
<td>Reagent B (E-4-P)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent C (F-6-P)</td>
<td>0.10</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent E (ß-NADH)</td>
<td>0.15</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent D (MgCl₂)</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Reagent F (a-GDH/TPI)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 25°C. Monitor the A₃₄₀nm 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 $A_{340\text{nm}}$ for approximately 5 minutes. Obtain the $r A_{340\text{nm}}$/minute using the maximum linear rate for both the Test and Blank.
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CALCULATIONS:

Units/ml enzyme = \frac{(r_{A_{340\text{nm}}/\text{min Test}} - r_{A_{340\text{nm}}/\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 β-NADH at 340 nm
0.1 = Volume (in milliliter) of transaldolase used

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

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 α-glycerophosphate dehydrogenase/triosephosphate isomerase, 0.025 - 0.050 units transaldolase.

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 β-nicotinamide adenine dinucleotide, 0.01 mg of a-glycerophosphate dehydrogenase/triosephosphate isomerase, 0.025 - 0.050 units transaldolase.

REFERENCE:


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.