Enzymatic Assay of ALDOLASE
(EC 4.1.2.13)

PRINCIPLE:

Fructose 1,6-Diphosphate + H_2O $\xrightarrow{\text{Aldolase}}$ G 3-P + DHAP

G 3-P $\xrightarrow{\text{TPI}}$ DHAP

2 DHAP + 2 β-NADH $\xrightarrow{\alpha$-GDH} 2 a-Glycerophosphate + 2 β-NAD

Abbreviations used:
G 3-P = Glyceraldehyde 3-Phosphate
DHAP = Dihydroxyacetone 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 = 7.4, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Tris HCl Buffer, pH 7.4 at 25°C.
(Prepare 250 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.4 at 25°C with 1 M HCl.)

B. 58 mM Fructose 1,6-Diphosphate Solution (F 1,6-DP)
(Prepare 1 ml in deionized water using D-Fructose 1,6-Diphosphate, Tetra(cyclohexylammonium) Salt, Sigma Prod. No. F-0752.)

C. 4.0 mM β-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β-NADH)
(Prepare 2 ml in cold deionized water using β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129 or 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 deionized water.)

D. α-Glycerophosphate Dehydrogenase/Triosephosphate Isomerase Enzyme Solution (α-GDH/TPI)
(Immediately before use, prepare a solution containing 50 α-GDH units/ml of α-Glycerophosphate Dehydrogenase/Triosephosphate Isomerase, Sigma Prod. No. G-6755, in cold deionized water.)
Enzymatic Assay of ALDOLASE\(^{1}\)
(EC 4.1.2.13)

REAGENTS: (continued)

E. Aldolase Enzyme Solution
(Immediately before use, prepare a solution containing 0.25 - 0.50 unit/ml of Aldolase in cold
Reagent A.)

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>Reagent A (Buffer)</td>
<td>2.60</td>
<td>2.60</td>
</tr>
<tr>
<td>Reagent B (F 1,6-DP)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (β-NADH)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent D (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 \(A_{340\text{nm}}\) until constant, using a suitably
thermostatted spectrophotometer. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>-----</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent E (Enzyme Solution)</td>
<td>0.10</td>
<td>-----</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the decrease in \(A_{340\text{nm}}\) for approximately 5 minutes. Obtain
the \(r_{A_{340\text{nm}}/\text{min}}\) using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(r_{A_{340\text{nm}}/\text{min Test} - r_{A_{340\text{nm}}/\text{min Blank}})(3)(df)}{(2)(6.22)(0.1)}
\]

3 = Total volume (in milliliters) of assay
df = Dilution factor
2 = 2 moles of β-NADH converted to 2 moles of β-NAD per mole of Fructose 1,6-Diphosphate
6.22 = Millimolar extinction coefficient of β-NADH at 340 nm
0.1 = Volume (in milliliter) of enzyme used
Enzymatic Assay of ALDOLASE\(^{1}\)  
(EC 4.1.2.13)

**CALCULATIONS:** (continued)

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

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

**UNIT DEFINITION:**

One unit will convert 1.0 \(\mu\)mole of fructose 1,6-diphosphate to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate per minute at pH 7.4 at 25\(^\circ\)C.

**FINAL ASSAY CONCENTRATION:**

In a 3.00 ml reaction mix, the final concentrations are 90 mM Tris, 1.9 mM fructose 1,6-diphosphate, 0.13 mM \(\beta\)-nicotinamide adenine dinucleotide, 5 units \(\alpha\)-glycerophosphate dehydrogenase/triosephosphate isomerase (based on \(\alpha\)-glycerophosphate dehydrogenase units) and 0.025 - 0.050 unit aldolase.

**REFERENCE:**


**NOTES:**

1. This enzyme assay is not to be used to assay Aldolase, from Staphylococcus aureus, Sigma Prod. No. A-2548, Aldolase, insoluble enzyme attached to polyacrylamide from Rabbit Muscle, Sigma Prod. No. A-1386, and Aldolase from Baker's Yeast, Sigma Prod. No. A-9562.

2. \(\alpha\)-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\(^\circ\)C.

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\(^\circ\)C.

4. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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