Enzymatic Assay of α-Glycerophosphate Dehydrogenase (EC 1.1.1.8)

PRINCIPLE:

\[ \text{DHAP} + \beta\text{-NADH} \xrightarrow{\alpha\text{-Glycerophosphate Dehydrogenase}} \text{α-Glycerophosphate} + \beta\text{-NAD} \]

Abbreviations used:
- DHAP = Dihydroxyacetone Phosphate
- 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. 300 mM Triethanolamine HCl Buffer, pH 7.4 at 25°C
   (Prepare 100 ml in deionized water using Triethanolamine Hydrochloride. Adjust to pH 7.4 at 25°C with 1 M NaOH.)

B. 76 mM Dihydroxyacetone Phosphate Solution (DHAP)
   (Prepare 1 ml in deionized water using Dihydroxyacetone Phosphate, Lithium Salt.)

C. 4 mM β-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β-NADH)
   (Prepare 1 ml in Reagent A using β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt.)

D. α-Glycerophosphate Dehydrogenase Enzyme Solution
   (Immediately before use, prepare a solution containing 0.15 - 0.30 unit/ml of α-Glycerophosphate Dehydrogenase in cold Reagent A.)
Enzymatic Assay of \( \alpha \)-GLYCEROPHOSPHATE DEHYDROGENASE
(EC 1.1.1.8)

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>2.70</td>
<td>2.70</td>
</tr>
<tr>
<td>Reagent B (DHAP)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (( \beta )-NADH)</td>
<td>0.10</td>
<td>0.10</td>
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</tbody>
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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>
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</table>
| Reagent D (Enzyme Solution) | 0.10 | -----
| Reagent A (Buffer)          | ---- | 0.10  |

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

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{r A_{340\text{nm}}/\text{min Test} - r A_{340\text{nm}}/\text{min Blank})(3)(df)}{(6.22)(0.10)}
\]

- 3 = Volume (in milliliter) of assay
- df = Dilution factor
- 6.22 = Millimolar extinction coefficient of \( \beta \)-NADH at 340 nm
- 0.1 = Volume (in milliliter) of enzyme used

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

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

UNIT DEFINITION:

One unit will convert 1.0 \( \mu \)mole of dihydroxyacetone phosphate to \( \alpha \)-glycerophosphate per minute at pH 7.4 at 25°C.
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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 290 mM triethanolamine, 2.5 mM dihydroxyacetone phosphate, 0.13 mM β-nicotinamide adenine dinucleotide reduced form and 0.015 - 0.03 unit α-glycerophosphate dehydrogenase.

REFERENCES:
