Enzymatic Assay of PYRUVATE DECARBOXYLASE
(EC 4.1.1.1)

PRINCIPLE:

Pyruvate $\text{PDC} \rightarrow \text{Acetaldehyde} + \text{CO}_2$

Acetaldehyde + $\beta$-NADH $\text{ADH} \rightarrow \text{Ethanol} + \beta$-NAD

Abbreviations used:
PDC = Pyruvate Decarboxylase
$\beta$-NADH = $\beta$-Nicotinamide Adenine Dinucleotide, Reduced Form
ADH = Alcohol Dehydrogenase
$\beta$-NAD = $\beta$-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS:  $T = 25^\circ \text{C}$, $\text{pH} 6.0$, $A_{340\text{nm}}$, Light path = 1 cm

METHOD:  Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 200 mM Citrate Buffer, pH 6.0 at 25°C
   (Prepare 100 ml in deionized water, using Citric Acid, Free Acid, Anhydrous. Adjust
to pH 6.0 at 25°C with 1 M NaOH.)

B. 1 M Sodium Pyruvate Solution (Pyr)
   (Prepare 5 ml in deionized water using Pyruvic Acid, Sodium Salt.)

C. 6.4 mM $\beta$-Nicotinamide Adenine Dinucleotide, Reduced Form, Solution ($\beta$-NADH)
   (Dissolve the contents of one 5 mg vial of $\beta$- Nicotinamide
   Adenine Dinucleotide, Reduced Form, Disodium, in the appropriate volume of
deionized water.)

D. Alcohol Dehydrogenase Enzyme Solution (ADH)
   (Immediately before use, prepare a solution containing
200 units/ml of Alcohol Dehydrogenase,
in cold deionized water.)
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REAGENTS:  (continued)

E. Pyruvate Decarboxylase Enzyme Solution (PDC)  
Immediately before use, prepare a solution containing 0.3 - 0.6 unit/ml of Pyruvate Decarboxylase 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>
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<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.70</td>
<td>2.90</td>
</tr>
<tr>
<td>Reagent B (Pyr)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (β-NADH)</td>
<td>0.05</td>
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<tr>
<td>Reagent D (ADH)</td>
<td>0.05</td>
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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:

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<tbody>
<tr>
<td>Reagent E (PDC)</td>
<td>0.10</td>
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</tbody>
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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.

CALCULATIONS:

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

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 enzyme used

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

UNIT DEFINITION:

One unit will convert 1.0 µmole of pyruvate to
acetaldehyde per minute at pH 6.0 at 25°C.
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FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 187 mM citric acid, 33 mM sodium pyruvate, 0.11 mM ß-nicotinamide adenine dinucleotide, reduced form, 10 units alcohol dehydrogenase, and 0.03 - 0.06 unit pyruvate decarboxylase.

REFERENCES:


NOTES:

1. Alcohol Dehydrogenase Unit Definition: One unit will convert 1.0 µmole of ethanol to acetaldehyde per minute at pH 8.8 at 25°C.

2. This assay is based on the cited reference.