Enzymatic Assay of PYRUVATE CARBOXYLASE  
(EC 6.4.1.1)

PRINCIPLE:

\[
\text{PC} \quad \text{Pyruvate} + \text{ATP} + \text{HCO}_3^- \rightarrow \text{Oxalacetate} + \text{ADP} + \text{P}_i \\
\text{AcCoA}
\]

\[
\text{MDH} \quad \text{Oxalacetate} + \beta\text{-NADH} \rightarrow \text{Malate} + \beta\text{-NAD}
\]

Abbreviations used:
ATP = Adenosine 5'-Triphosphate
PC = Pyruvate Carboxylase
AcCoA = Acetyl Coenzyme A
ADP = Adenosine 5'-Diphosphate
\(P_i\) = Inorganic Phosphate
\(\beta\text{-NADH}\) = \(\beta\text{-Nicotinamide Adenine Dinucleotide, Reduced Form}\)
MDH = Malic Dehydrogenase
\(\beta\text{-NAD}\) = \(\beta\text{-Nicotinamide Adenine Dinucleotide, Oxidized Form}\)

CONDITIONS: \(T = 30^\circ\text{C}, \text{pH} = 7.8, A_{340nm}, \text{Light path} = 1\text{ cm}\)

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 135 mM Triethanolamine Buffer with 7 mM Magnesium Sulfate, 9 mM Pyruvic Acid, and 0.15% (w/v) Bovine Serum Albumin, pH 8.0 at 30°C (Substrate)  

B. 0.3 mM Acetyl Coenzyme A Solution  
(Prepare 10 ml in deionized water using Acetyl Coenzyme A, Sodium Salt, Sigma Prod. No. A-2056. PREPARE FRESH.)
Enzymatic Assay of PYRUVATE CARBOXYLASE
(EC 6.4.1.1)

REAGENTS: (continued)

C. Malic Dehydrogenase Enzyme Solution (AcCoA/MDH)
(Immediately before use, add 150 units of Malic Dehydrogenase, Sigma Prod. No. M-9004 to 5 ml of Reagent B. Bring the solution to a total volume of 10.0 ml with deionized water.)

D. 100 mM Triethanolamine Buffer with 30 mM Adenosine
5'-Triphosphate and 450 mM Potassium Bicarbonate, pH 8.0 at 30°C (ATP/KHCO₃)

E. 2.6 mM β-Nicotinamide Adenine Dinucleotide, Reduced Form (β-NADH)
(Prepare 10 ml in deionized water using β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129.)

F. 50 mM Tris HCl Buffer with 50% (v/v) Glycerol, 2 mM Magnesium Acetate, and 1 mM Ethylenediaminetetraacetic Acid, pH 7.4 at 30°C (Enz Dil)

G. Pyruvate Carboxylase Enzyme Solution (PC)
(Immediately before use, prepare a solution containing approximately 30 - 90 units/ml of Pyruvate Carboxylase in cold Reagent F.)

PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Substrate)</td>
<td>20.00</td>
</tr>
<tr>
<td>C (AcCoA/MDH)</td>
<td>5.00</td>
</tr>
<tr>
<td>E (β-NADH)</td>
<td>2.50</td>
</tr>
</tbody>
</table>

Mix by swirling. Adjust to pH 7.8 at 30°C if necessary, with either 1 M HCl or 1 M KOH.
Enzymatic Assay of PYRUVATE CARBOXYLASE
(EC 6.4.1.1)

PROCEDURE:

Pipette (in milliliters) the following reagents into a suitable container:

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Cocktail</td>
<td>2.90</td>
</tr>
<tr>
<td>Reagent G (PC)</td>
<td>0.005</td>
</tr>
<tr>
<td>Reagent F (Enz Dil)</td>
<td>-----</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 30°C. Monitor the $A_{340nm}$ until constant using a suitably thermostatted spectrophotometer. Record the $\Delta A_{340nm}$/minute of the Test. Then add:

| Reagent D (ATP/KHCO$_3$) | 0.10 | 0.10 |

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

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(3.005)(\text{df})}{(6.22)(0.005)}
\]

3.005 = Total volume (in milliliters) of the assay
\(\text{df} = \text{Dilution factor}\)
6.22 = Millimolar extinction coefficient of $\beta$-NADH at 340 nm
0.005 = Volume (in milliliter) of pyruvate carboxylase used in the assay

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

UNIT DEFINITION:

One unit will convert 1.0 µmole of pyruvate and CO$_2$ to oxalacetate per minute at pH 7.8 at 30°C.
Enzymatic Assay of PYRUVATE CARBOXYLASE
(EC 6.4.1.1)

FINAL CONCENTRATIONS:

In a 3.005 ml reaction mix, the final concentrations are 134 mM triethanolamine, 5 mM magnesium sulfate, 7 mM pyruvic acid, 0.12% (w/v) bovine serum albumin, 0.23 mM β-nicotinamide adenine dinucleotide, reduced form, 0.05 mM acetyl coenzyme A, 2.63 units malic dehydrogenase, 1 mM adenosine 5'-triphosphate, 15 mM potassium bicarbonate, 0.05% (v/v) glycerol, 0.002 mM magnesium acetate, 0.001 mM ethylenediaminetetraacetic acid, 0.05 mM Tris, 0.15 - 0.45 unit pyruvate carboxylase.

REFERENCE:


NOTES:

1. Lactic Dehydrogenase is the principle contaminant that may interfere with the assay for pyruvate carboxylase. If the ΔA_340nm/min is not zero, it must be subtracted from the ΔA_340nm/min for the Test after the addition of Reagent D (ATP/KHCO_3).

2. Malic Dehydrogenase Unit Definition: One unit will convert 1.0 µmole of oxalacetate and β-NADH to L-malate and β-NAD per minute at pH 7.5 at 25°C.

3. This assay is based on the cited reference.

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

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