Enzymatic Assay of MALIC ENZYME
(E.C. 1.1.1.40)

**PRINCIPLE:**

\[
\text{Malic Enzyme} \\
\text{L-Malate + } \beta\text{-NADP} \rightarrow \text{Pyruvate + CO}_2 + \beta\text{-NADPH}
\]

Abbreviations used:
\(\beta\text{-NADP} = \beta\text{-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form}\)
\(\beta\text{-NADPH} = \beta\text{-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form}\)

**CONDITIONS:** T = 25°C, pH = 7.4, A
340
, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

A. 100 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. 100 mM L-Malic Acid Solution (Malic Acid)  
(Prepare 5 ml in deionized water using L(-)Malic Acid, Free Acid.)

C. 20 mM \(\beta\text{-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form, Solution (NADP)}\)  
(Prepare 2 ml in deionized water using \(\beta\text{-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt or }\beta\text{-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt.}\))

D. 20 mM Manganese Chloride Solution (MnCl\(_2\))  
(Prepare 25 ml in deionized water using Manganese Chloride, Tetrahydrate.)

E. Malic Enzyme Solution  
(Immediately before use, prepare a solution containing 0.25 - 0.50 unit/ml of Malic Enzyme in cold deionized water.)
Enzymatic Assay of MALIC ENZYME
(E.C. 1.1.1.40)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Reagent B (Malic Acid)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (NADP)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent D (MnCl₂)</td>
<td>0.75</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 25°C. Monitor the A₃₄₀ nm until constant, using a suitably thermostatted spectrophotometer. Then add:

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

Immediately mix by inversion and monitor the increase in A₃₄₀ nm for approximately 5-10 minutes. Obtain the ΔA₃₄₀ nm/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

3 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of β-NADPH 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 µmole of L-malate and NADP to pyruvate, CO₂ and NADPH per minute at pH 7.4 at 25°C.
Enzymatic Assay of MALIC ENZYME  
(E.C. 1.1.1.40)

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 67 mM triethanolamine, 3.3 mM L-malic acid, 0.3 mM β-nicotinamide adenine dinucleotide phosphate, 5.0 mM manganese chloride and 0.025 - 0.050 unit malic enzyme.

REFERENCE:


NOTES:

1. This assay is based on the cited reference.
2. Where our Product or Stock numbers are specified, equivalent reagents may be substituted.

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