Enzymatic Assay of CITRATE SYNTHASE  
(EC 4.1.3.7)

**PRINCIPLE:**

\[
\text{L-Malate} + \beta\text{-NAD} \xrightarrow{\text{MDH}} \text{Oxalacetate} + \beta\text{-NADH}
\]

\[
\text{Acetyl CoA} + \text{Oxalacetate} \xrightarrow{\text{CS}} \text{Citrate} + \text{CoA-SH}
\]

Abbreviations used:

MDH = Malic Dehydrogenase  
\(\beta\text{-NAD} = \beta\text{-Nicotinamide Adenine Dinucleotide, Oxidized Form}\)  
\(\beta\text{-NADH} = \beta\text{-Nicotinamide Adenine Dinucleotide, Reduced Form}\)  
CoA = Coenzyme A  
CS = Citrate Synthase

**CONDITIONS:**  
\(T = 37^\circ C, \text{pH} = 8.0, A_{340nm}, \text{Light path} = 1 \text{ cm}\)

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

A. 100 mM Tris Buffer, pH 8.0 at 37°C  
(Prepare 100 ml in deionized water using Trizma Base, Prod. No. T-1503. Adjust to pH 8.0 at 37°C with 1 M HCl.)

B. 100 mM L-Malic Acid  
(Prepare 10 ml in Reagent A using L(-)Malic Acid, Monosodium Salt, Prod. No. M-1125.)

C. 50 mM \(\beta\text{-Nicotinamide Adenine Dinucleotide, Oxidized Form Solution (\beta\text{-NAD})}\)  
(Prepare 2 ml in deionized water using \(\beta\text{-Nicotinamide Adenine Dinucleotide, from Yeast, Prod. No. N-7004.}\)

D. 2.0 mM Acetyl Coenzyme A Solution (Acetyl CoA)  
(Prepare 2.0 ml in Reagent A using Acetyl Coenzyme A, Sodium Salt, Prod. No. A-2056.)

E. Malic Dehydrogenase Enzyme Solution (MDH)  
(Prepare 1.0 ml in Reagent A containing approximately 560 units/ml Malic Dehydrogenase, Prod. No. M-2634.)
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REAGENTS: (continued)

F. Citrate Synthase Enzyme Solution (CS)  
(Prepare a solution containing 0.2 - 0.4 units of Citrate Synthase in cold Reagent A.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th>Reagent A (Buffer)</th>
<th>7.50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (L-Malic Acid)</td>
<td>2.00</td>
</tr>
<tr>
<td>Reagent C (β-NAD)</td>
<td>1.00</td>
</tr>
<tr>
<td>Reagent D (Acetyl CoA)</td>
<td>2.00</td>
</tr>
<tr>
<td>Reagent E (MDH)</td>
<td>1.00</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>15.50</td>
</tr>
</tbody>
</table>

Mix and adjust to pH 8.0 at 37°C with 1 M HCl or 1 M NaOH, if necessary.

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

<table>
<thead>
<tr>
<th>Reaction Cocktail</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.90</td>
<td>2.90</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 37°C. Monitor the A_{340nm} until constant using a suitably thermostatted spectrophotometer. Then add:

<table>
<thead>
<tr>
<th>Reagent F (CS)</th>
<th>0.10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>------</td>
</tr>
</tbody>
</table>

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

CALCULATIONS:

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

3 = Volume (in milliliters) of the assay

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

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

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

UNIT DEFINITION:

One unit will form 1.0 µmole of citrate from oxalacetate and acetyl CoA per minute at pH 8.0 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 3.0 ml reaction mix, the final concentrations are 46 mM Tris, 6.7 mM L-malic acid, 1.7 mM β-nicotinamide adenine dinucleotide, 0.13 mM acetyl CoA, 56 units malic dehydrogenase, and 0.02 - 0.04 unit citrate synthase.

REFERENCE:


NOTES:

1. This assay is based on the cited reference.

2. 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.