**Enzymatic Assay of ISOCITRATE LYASE (EC 4.1.3.1)**

**PRINCIPLE:**

\[ \text{Isocitrate} \xrightarrow{\text{Isocitrate Lyase}} \text{Succinate + Glyoxylate} \]
\[ \text{Glyoxylate + Phenylhydrazine} \rightarrow \text{Phenylhydrazine} \]
\[ \text{Glyoxylate} \]

**CONDITIONS:** \( T = 30^\circ C, \ \text{pH} = 6.8, \ A_{324nm}, \ \text{Light Path} = 1 \ \text{cm} \)

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

A. 50 mM Imidazole Buffer, pH 6.8 at 30°C
   (Prepare 100 ml in deionized water using Imidazole. Adjust to pH 6.8 at 30°C with 1 M HCl.)

B. 50 mM Magnesium Chloride Solution (MgCl₂)
   (Prepare 10 ml in deionized water using Magnesium Chloride, Hexahydrate.)

C. 10 mM Ethylenediaminetetraacetic Acid Solution (EDTA)
   (Prepare 10 ml in deionized water using Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate.)

D. 40 mM Phenylhydrazine HCl Solution (Phenylhydrazine)
   (Prepare 10 ml in deionized water using Phenylhydrazine Hydrochloride.)

E. 10 mM DL-Isocitric Acid Solution (Isocitrate)
   (Prepare 10 ml in deionized water using DL-Isocitric Acid, Trisodium Salt.)

F. Isocitrate Lyase Enzyme Solution
   (Immediately before use, prepare a solution containing 0.05 – 0.07 unit/ml of Isocitrate Lyase in cold Reagent A.)
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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>A (Buffer)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>B (MgCl(_2))</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>C (EDTA)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>D (Phenylhydrazine)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>E (Isocitrate)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 30°C. Monitor the A\(_{324\text{nm}}\) 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>F (Enzyme Solution)</td>
<td>0.10</td>
<td>------</td>
</tr>
<tr>
<td>A (Buffer)</td>
<td>------</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the increase in A\(_{324\text{nm}}\) for approximately 5 minutes. Obtain the \(r\) A\(_{324\text{nm}}\)/minute using the maximum linear rate for both the Test and Blank.

CONDITIONS:

\[ \text{Units/ml enzyme} = \frac{(r\ A_{324\text{nm}}/\text{min Test} - r\ A_{324\text{nm}}/\text{min Blank})(1)(df)}{(16.8)(0.1)} \]

1 = Volume (in milliliter) of assay
df = Dilution factor
16.8 = Millimolar extinction coefficient of Phenylhydrazine
Glyoxylate at 324 nm
0.1 = Volume (in milliliter) of enzyme used

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

UNIT DEFINITION:

One unit catalyzes the formation of 1 µmole of glyoxylate per minute at pH 6.8 at 30°C.
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FINAL ASSAY CONCENTRATION:

In a 1.00 ml reaction mix, the final concentrations are 30 mM imidazole, 5 mM magnesium chloride, 1 mM ethylenediaminetetraacetic acid, 4 mM phenylhydrazine, 1 mM DL-isocitric acid, and 0.005 - 0.007 unit isocitrate lyase.

REFERENCE:


NOTES:

1. This assay is based on the cited reference.

This procedure is for informational purposes.