Enzymatic Assay of ISOCITRATE LYASE
(EC 4.1.3.1)

PRINCIPLE:

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

\[
\text{Glyoxylate} + \text{Phenylhydrazine} \rightarrow \text{Phenylhydrazine}
\]

\[
\text{Glyoxylate}
\]

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, Sigma Prod. No. I-0125. Adjust to pH 6.8 at 30°C with 1 M HCl.)

B. 50 mM Magnesium Chloride Solution (MgCl\(_2\))
   (Prepare 10 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)

C. 10 mM Ethylenediaminetetraacetic Acid Solution (EDTA)
   (Prepare 10 ml in deionized water using Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS.)

D. 40 mM Phenylhydrazine HCl Solution (Phenylhydrazine)
   (Prepare 10 ml in deionized water using Phenylhydrazine Hydrochloride, Sigma Prod. No. P-6926.)

E. 10 mM DL-Isocitric Acid Solution (Isocitrate)
   (Prepare 10 ml in deionized water using DL-Isocitric Acid, Trisodium Salt, Sigma Prod. No. I-1252.)

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 $rA_{324\text{nm}}$/minute using the maximum linear rate for both the Test and Blank.

CONDITIONS:

$$\text{Units/ml enzyme} = \frac{(rA_{324\text{nm}}/\text{min Test} - rA_{324\text{nm}}/\text{min Blank})(1)(df)}{16.8(0.1)}$$

1 = Volume (in milliliter) of assay  
df = Dilution factor  
16.8 = Millimolar extinction coefficient of Glyoxylate at 324 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 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.

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.