Enzymatic Assay of OXALATE DECARBOXYLASE  
(EC 4.1.1.2)

PRINCIPLE:

\[
\text{Oxalate} \xrightarrow{\text{Oxalate Decarboxylase}} \text{Formate} + \text{CO}_2
\]

CONDITIONS:  \( T = 37\,^\circ\text{C}, \text{pH} \, 3.0 \)

METHOD:  Manometric Assay using Warburg Flasks

Reagents:

A. 100 mM Potassium Citrate Buffer, pH 3.0 at 37°C  
(Prepare 100 ml in deionized water using Citric Acid,  
Tripotassium Salt, Sigma Prod. No. C-8385. Adjust to  
pH 3.0 at 37°C with 1 M HCl.)

B. 100 mM Oxalic Acid Solution, pH 3.0 at 37°C (Ox Acid)  
(Prepare 10 ml in Reagent A using Oxalic Acid,  
Dipotassium Salt, Monohydrate, Sigma Prod. No. O-0501.  
Adjust the pH to 3.0 with 1 M HCl or 1 M KOH, if  
necessary.)

C. Oxalate Decarboxylase Enzyme Solution  
(Immediately before use, prepare a solution containing  
2 units/ml of Oxalate Decarboxylase in cold deionized  
water.)

PROCEDURE:

Pipette (in milliliters) the following reagents into  
Warburg flasks:

<table>
<thead>
<tr>
<th>Main Chamber</th>
<th>Thermo-barometer</th>
<th>Enzyme Flask</th>
<th>Blank</th>
<th>Test</th>
<th>Substrate</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.90</td>
<td>2.70</td>
<td>2.70</td>
<td>2.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reagent C (Enzyme Solution)</td>
<td>________</td>
<td>_______</td>
<td>0.20</td>
<td>0.20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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PROCEDURE: (continued)

<table>
<thead>
<tr>
<th>Side Arm</th>
<th>Thermo-</th>
<th>Enzyme</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>barometer Flask</td>
<td>Blank</td>
<td>Test</td>
</tr>
<tr>
<td>Reagent B (Ox Acid)</td>
<td>------</td>
<td>------</td>
<td>0.10</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>0.10</td>
<td>0.10</td>
<td>------</td>
</tr>
</tbody>
</table>

Be sure to confirm the stability of the pressure with the flask sealed off before proceeding with the assay. This is to ensure temperature equilibrium and the absence of leaks in the flask. Equilibrate the closed system for 30 minutes at 37°C.

The enzyme activity is determined by calculation of the rate of production of CO$_2$ at 37°C. The reaction rate should be linear for about 20 minutes.

CALCULATIONS:

\[
\text{Units} = \frac{(C)(K)(df)}{\text{ml Oxalate Decarboxylase}} = 22.4 \frac{l}{\text{mole}}\times\frac{1}{(\text{ml oxalate decarboxylase})}
\]

- \(C\) = mm of CO$_2$ gas evolved/minute$^2$
- \(K\) = Warburg flask constant$^3$ in µl/mm
- 22.4 l = Volume gas occupies under STP conditions

UNIT DEFINITIONS:

One unit will convert 1.0 µmole of oxalate to formate and CO$_2$ per minute at pH 3.0 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 93 mM potassium citrate, 3.3 mM oxalic acid, and 0.4 unit oxalate decarboxylase.

REFERENCE:

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REFERENCE:

Shimazono, H. and Hayaishi, O., (1957) Journal of Biological Chemistry 227, 151-159

NOTES:

1. The tests are done in triplicate, since it is common for the flasks to have small leaks.

2. The mm of CO₂ gas evolved (C) is corrected for any temperature and barometric changes (Thermobarometer) during the experiment and also for the Substrate Blank and Enzyme Blank:

   \[ \text{mm CO}_2 \text{ corrected} = \text{mm CO}_2 \text{ measured Test} - \text{mm CO}_2 \text{ measured for [Thermobarometer + Substrate Blank + Enzyme Blank]} \]

Values of the corrected mm CO₂ produced are plotted versus time. The best straight line is drawn not necessarily through the origin. The slope, \( C = \frac{\text{mm CO}_2}{\text{time}} \) is obtained.

3. The flask constant, \( K \), is calculated according to the equation:

   \[ K = \frac{\{(V_g) \left( \frac{273}{T} \right)^2 + V_\ell \ a\}}{P_o} \]

   where

   \( P_o \) = Standard pressure as mm of manometer fluid
   \( V_g \) = Volume (in milliliters) of gas in flask and manometer
   \( V_\ell \) = Volume (in milliliters) of liquid in flask
   \( T \) = Absolute temperature
   \( a \) = Solubility of gas; (for CO₂ at 37°C, \( a = 0.57 \))
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NOTES: (continued)

3. The flask constant, K, must be calculated for each Warburg flask used, as described in Umbreit, W.W., Burris, R.H. and Stauffer, J.F. (1951).

4. This assay is based on the cited references.

5. 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.