Enzymatic Assay of OXALATE DECARBOXYLASE
(EC 4.1.1.2)

PRINCIPLE:

Oxalate $\xrightarrow{\text{Oxalate Decarboxylase}}$ Formate + CO_2

CONDITIONS: T = 37°C, 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:

Main Chamber	Thermo-barometer Flask	Enzyme Blank	Test[1]	Substrate Blank
Reagent A (Buffer)	2.90	2.70	2.70	2.90
Reagent C (Enzyme Solution)	------	------	0.20	0.20

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PROCEDURE: (continued)

Side Arm	Thermo-barometer Flask	Enzyme Blank	Test[1]	Substrate Blank
Reagent B (Ox Acid)	------	------	0.10	0.10
Deionized Water	0.10	0.10	------	------

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.[2] The reaction rate should be linear for about 20 minutes.

CALCULATIONS:

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

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:

Umbreit W.W., Burris R.H., and Stauffer, J.F. (1951) in *Manometric Techniques and Tissue Metabolism,* Burgess Publishing Co. Minneapolis, MN

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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 CO_2 gas evolved (C) is corrected for any temperature and barometric changes (Thermobarometer) during the experiment and also for the Substrate Blank and Enzyme Blank:

   mm $CO_{2\ corrected}$ = mm $CO_{2\ measured}$ Test - mm $CO_{2\ measured}$ for [Thermobarometer + Substrate Blank + Enzyme Blank]

   Values of the corrected mm CO_2 produced are plotted versus time. The best straight line is drawn not necessarily through the origin. The slope, C = mm CO_2/time is obtained.

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

$$K = \frac{[(V_g)\ (\frac{273}{T}2) + V_f\ a]}{P_o}$$

   where

   P_o = Standard pressure as mm of manometer fluid
   V_g = Volume (in milliliters) of gas in flask and manometer
   V_f = Volume (in milliliters) of liquid in flask
   T = Absolute temperature
   a = Solubility of gas; (for CO_2 at 37°C, a = 0.57)

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.