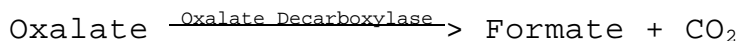


**Enzymatic Assay of OXALATE DECARBOXYLASE  
(EC 4.1.1.2)**

**PRINCIPLE:**



**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 <u>Flask</u>	Enzyme <u>Blank</u>	<u>Test<sup>1</sup></u>	Substrate <u>Blank</u>
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 <sup>1</sup>	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<sub>2</sub> at 37°C.<sup>2</sup> The reaction rate should be linear for about 20 minutes.

**CALCULATIONS:**

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

C = mm of CO<sub>2</sub> gas evolved/minute<sup>2</sup>

K = Warburg flask constant<sup>3</sup> in  $\mu\text{l}/\text{mm}$

22.4 l = Volume gas occupies under STP conditions

**UNIT DEFINITIONS:**

One unit will convert 1.0  $\mu\text{mole}$  of oxalate to formate and CO<sub>2</sub> 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<sub>2</sub> gas evolved (C) is corrected for any temperature and barometric changes (Thermobarometer) during the experiment and also for the Substrate Blank and Enzyme Blank:

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

Values of the corrected mm CO<sub>2</sub> produced are plotted versus time. The best straight line is drawn not necessarily through the origin. The slope, C = mm CO<sub>2</sub>/time is obtained.

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

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

where

P<sub>o</sub> = Standard pressure as mm of manometer fluid

V<sub>g</sub> = Volume (in milliliters) of gas in flask  
and manometer

V<sub>f</sub> = Volume (in milliliters) of liquid in flask

T = Absolute temperature

a = Solubility of gas; (for CO<sub>2</sub> 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.**