Enzymatic Assay of OXALATE DECARBOXYLASE (EC 4.1.1.2)

PRINCIPLE:

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

$$\text{Formate} + \text{ß-NAD} \xrightarrow{\text{Formate Dehydrogenase}} CO_2 + \text{ß-NADH}$$

Abbreviations used:
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form

CONDITIONS: T = 37°C, pH = 5.0, A_{340nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 100 mM Potassium Phosphate Solution
(Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Sigma Prod. No. P-5504.)

B. 100 mM Potassium Phosphate Buffer, pH 5.0 at 37°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 5.0 at 37°C with Reagent A.)

C. 200 mM Oxalic Acid Solution
(Prepare 100 ml in deionized water using Oxalic Acid, Dihydrate, Sigma Prod. No. O-0505.)

D. 200 mM Oxalate Substrate Solution (Oxal)
(Prepare 100 ml in deionized water using Oxalic Acid, Dipotassium Salt, Monohydrate, Sigma Prod. No. O-0501.[1] Adjust to pH 5.0 at 37°C with Reagent C.)

E. 150 mM Potassium Phosphate Solution
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379.)

REAGENTS: (continued)

F. 150 mM Potassium Phosphate Buffer, pH 7.5 at 37°C (Stop Buffer)
(Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Sigma Prod. No. P-5504. Adjust to pH 7.5 at 37°C with Reagent E.)

G. 57 mM ß-Nicotinamide Adenine Dinucleotide, Oxidized Form Solution (ß-NAD)
(Prepare 5 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide, Lithium Salt, Sigma Prod. No. N-7132.)

H. Formate Dehydrogenase Enzyme Solution (FDH)
(Immediately before use, prepare a solution containing 40 units/ml of Formate Dehydrogenase, Sigma Prod. No. F-8649, in cold deionized water.)

I. Oxalate Decarboxylase Enzyme Solution (Ox Decarb)
(Immediately before use, prepare a solution containing 0.5 - 1.0 unit/ml of Oxalate Decarboxylase in cold Reagent B.)

PROCEDURE:

Step 1:

Pipette (in milliliters) the following reagents into suitable containers:

	Test	Blank
Reagent B (Buffer)	0.30	0.30
Reagent I (Ox Decarb)	0.10	------

Mix by swirling and equilibrate to 37°C. Then add:

Reagent D (Oxal)	0.20	0.20

Immediately mix by swirling and incubate at 37°C for exactly 2 minutes. Then add:

Reagent F (Stop Buffer)		2.00
		2.00
Reagent I (Ox Decarb)	------	0.10

Mix by swirling.

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PROCEDURE: (continued)

Step 2:

Transfer the Test and Blank solutions into suitable cuvettes. Then add:

	Test	Blank
Reagent G (ß-NAD)	0.20	0.20

Mix by inversion and equilibrate to 37°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Record the initial A_{340nm} for both the Test and Blank. Then add:

Reagent H (FDH)	0.20	0.20

Immediately mix by inversion and record the increase in A_{340nm} until constant (for approximately 5 minutes). Obtain the final A_{340nm} for both the Test and Blank.

CALCULATIONS:

$$\Delta A_{340nm}\ \text{Test} = A_{340nm}\ \text{Test Final} - A_{340nm}\ \text{Test Initial}$$

$$\Delta A_{340nm}\ \text{Blank} = A_{340nm}\ \text{Blank Final} - A_{340nm}\ \text{Blank Initial}$$

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}\ \text{Test} - \Delta A_{340nm}\ \text{Blank})(3)(df)}{(6.22)(0.1)(2)}$$

3 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
0.1 = Volume (in milliliter) of enzyme used
2 = Time (in minutes) as per the Unit Definition

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

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UNIT DEFINITION:

One unit will convert 1.0 µmole of oxalate to formate and CO_2 per minute at pH 5.0 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 0.60 ml reaction mix, the final concentrations are 67 mM potassium phosphate, 67 mM oxalate, and 0.05 - 0.10 unit oxalate decarboxylase.

REFERENCE:

Bergmeyer, H.U., Grassl, M., and Walter, H.-E. (1983) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) 3rd ed., Volume II, 261-262, Verlag Chemie, Deerfield Beach, FL

NOTES:

1. Do not use the sodium salt of this product, as it will inhibit the reaction.

2. Formate Dehydrogenase Unit Definition: One unit will oxidize 1.0 µmole of formate to CO_2 per minute in the presence of ß-NAD at pH 7.6 at 37°C.

3. This assay is based on the cited reference.

4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.