

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

PRINCIPLE:

Oxalate $\xrightarrow{\text{Oxalate Decarboxylase}}$ Formate + CO₂

Formate + β-NAD $\xrightarrow{\text{Formate Dehydrogenase}}$ CO₂ + β-NADH

Abbreviations used:

β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form

β-NADH = β-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.)

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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 β -Nicotinamide Adenine Dinucleotide, Oxidized Form Solution (β -NAD)
(Prepare 5 ml in deionized water using β -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:

	<u>Test</u>	<u>Blank</u>
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
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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:

	<u>Test</u>	<u>Blank</u>
Reagent G (β-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
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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:

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

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

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

3 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of β-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 μ mole of oxalate to formate and CO₂ 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 μ mole of formate to CO₂ per minute in the presence of β -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.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.