Enzymatic Assay of OXALACETATE DECARBOXYLASE
(EC 4.1.1.3)

PRINCIPLE:

Oxalacetic Acid $\xrightarrow{\text{Oxalacetate Decarboxylase}}$ Pyruvic Acid + CO_2

Pyruvic Acid + ß-NADH + H^+ $\xrightarrow{\text{LDH}}$ Lactic Acid + ß-NAD^+

Abbreviations used:
LDH = Lactate Dehydrogenase
ß-NAD = ß-Nicotinamide Adenine Dinucleotide
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form

CONDITIONS: T = 25°C, pH = 8.0, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Triethanolamine HCl pH 8.0 at 25°C (TEA Buffer)
(Prepare in deionized water using Triethanolamine HCl, Sigma Prod. No. T-1502. Adjust pH to 8.0 at 25°C with 1 M NaOH.)

B. 10 mM Tris HCl (Diluent)
(Prepare in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust pH to 8.0 at 25°C using 1 M HCl.)

C. 200 mM Tris HCl pH 9.0 at 25°C
(Prepare in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust pH to 9.0 using 5 M HCl.)

D. 10 mM Manganese Chloride Solution ($MnCl_2$)
(Prepare in deionized water using Manganese Chloride, Tetrahydrate, Sigma Prod. No. M-3634.)

E. 6.5 mM ß-Nicotinamide Adenine Dinucleotide, Reduced Form (NADH)
(Prepare in deionized water using ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Sodium Salt, Sigma Prod.
No. N-8129.)

Enzymatic Assay of OXALACETATE DECARBOXYLASE (EC 4.1.1.3)

REAGENTS: (continued)

F. Lactic Dehydrogenase Solution (LDH Solution)
(Immediately before use prepare a solution containing 550 units/ml Lactic Dehydrogenase, Sigma Prod. No. L-2500, using cold Reagent B as the diluent.)

G. 50 mM Oxalacetic Acid Solution (Substrate Solution)
(Immediately before use prepare a solution of Oxalacetic Acid, Sigma Prod. No. O-4126, in cold Reagent C. **PREPARE FRESH.**)

H. Oxalacetate Decarboxylase Solution
(Immediately before use prepare a solution containing 0.5 - 1.0 unit per ml in cold Reagent B.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes.

	Test	Blank
Reagent A (TEA Buffer)	0.90	0.90
Reagent D ($MnCl_2$)	0.10	0.10
Reagent E (ß-NADH)	0.10	0.10
Deionized Water	0.90	0.90
Reagent F (LDH Solution)	0.02	0.02
Reagent H (Enzyme Solution)	0.05	------
Reagent B (Diluent)	------	0.05

Mix by inversion and equilibrate to 25°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent F (Substrate)	0.10	0.10

Immediately mix by inversion and record the decrease in A_{340nm} from the linear portion of the curve. Obtain the ΔA_{340nm} for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(2.17)(df)}{(6.22)(0.05)}$$

CALCULATIONS: (continued)

2.17 = Total volume (in milliliter) of enzyme assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm.
0.05 = Volume (in milliliter) of enzyme used

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

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

UNIT DEFINITION:

One Unit will convert 1.0 µmole of oxalacetate to pyruvate and CO_2 per minute at pH 8.0 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 2.17 ml reaction mix, the final concentrations are 40 mM triethanolamine, 9.5 mM Trizma HCl, 0.5 mM manganese chloride, 0.3 mM ß-nicotinamide adenine dinucleotide, reduced form, 9.2 µg/ml lactic dehydrogenase, 2.3 mM oxalacetic acid, and 0.05 units per ml oxalacetate decarboxylase.

NOTES:

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