Enzymatic Assay of CITRATE SYNTHASE
(EC 4.1.3.7)

PRINCIPLE:

$_{L}$-Malate + ß-NAD $\xrightarrow{MDH}$ Oxalacetate + ß-NADH

Acetyl CoA + Oxalacetate $\xrightarrow{CS}$ Citrate + CoA-SH

Abbreviations used:
MDH = Malic Dehydrogenase
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
CoA = Coenzyme A
CS = Citrate Synthase

CONDITIONS: T = 37°C, pH = 8.0, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Tris Buffer, pH 8.0 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Prod. No. T-1503. Adjust to pH 8.0 at 37°C with 1 M HCl.)

B. 100 mM $_{L}$-Malic Acid
(Prepare 10 ml in Reagent A using $_{L}$(-)Malic Acid, Monosodium Salt, Prod. No. M-1125.)

C. 50 mM ß-Nicotinamide Adenine Dinucleotide, Oxidized Form Solution (ß-NAD)
(Prepare 2 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide, from Yeast, Prod. No. N-7004.)

D. 2.0 mM Acetyl Coenzyme A Solution (Acetyl CoA)
(Prepare 2.0 ml in Reagent A using Acetyl Coenzyme A, Sodium Salt, Prod. No. A-2056.)

E. Malic Dehydrogenase Enzyme Solution (MDH)
(Prepare 1.0 ml in Reagent A containing approximately 560 units/ml Malic Dehydrogenase, Prod. No. M-2634.)

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REAGENTS: (continued)

F. Citrate Synthase Enzyme Solution (CS)
(Prepare a solution containing 0.2 - 0.4 units of Citrate Synthase in cold Reagent A.)

PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

Reagent A (Buffer)	7.50
Reagent B (L-Malic Acid)	2.00
Reagent C (ß-NAD)	1.00
Reagent D (Acetyl CoA)	2.00
Reagent E (MDH)	1.00
Deionized Water	15.50

Mix and adjust to pH 8.0 at 37°C with 1 M HCl or 1 M NaOH, if necessary.

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

	Test	Blank
Reaction Cocktail	2.90	2.90

Mix by inversion and equilibrate to 37°C. Monitor the A_{340nm} until constant using a suitably thermostatted spectrophotometer. Then add:

Reagent F (CS)	0.10	------
Reagent A (Buffer)	------	0.10

Immediately mix by inversion and record the increase in A_{340nm} for approximately 5 minutes. Obtain the ΔA_{340nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

3 = Volume (in milliliters) of the assay
df = Dilution factor

6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
0.1 = 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 form 1.0 µmole of citrate from oxalacetate and acetyl CoA per minute at pH 8.0 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 3.0 ml reaction mix, the final concentrations are 46 mM Tris, 6.7 mM L-malic acid, 1.7 mM ß-nicotinamide adenine dinucleotide, 0.13 mM acetyl CoA, 56 units malic dehydrogenase, and 0.02 - 0.04 unit citrate synthase.

REFERENCE:

Srere, P.A. (1969), *Methods in Enzymology*, XIII, 1-11

NOTES:

1. This assay is based on the cited reference.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.