

**Enzymatic Assay of CITRATE LYASE
(EC 4.1.3.6)**

PRINCIPLE:

Citrate + H₂O Citrate Lyase > Oxalacetate + Acetate

Oxalacetate + β-NADH Malic Dehydrogenase > L-Malate + β-NAD

(Oxalacetate Oxalacetate Decarboxylase > Pyruvate + CO₂)¹

Pyruvate + β-NADH L-Lactic Dehydrogenase > L-Lactate + β-NAD

Abbreviations used:

β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form

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

CONDITIONS: T = 25°C, pH = 7.6, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Triethanolamine Buffer, pH 7.6 at 25°C
(Prepare 100 ml in deionized water using Triethanolamine Hydrochloride, Sigma Prod. No. T-1502. Adjust to pH 7.6 at 25°C with 1 M NaOH.)
- B. 29 mM Zinc Chloride Solution (ZnCl₂)
(Prepare 5 ml in deionized water using Zinc Chloride, Sigma Prod. No. Z-4875.)
- C. 14 mM β-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β-NADH)
(Prepare 1 ml in deionized water using β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129 or dissolve the contents of a 10 mg vial of β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-110, in the appropriate volume of deionized water. **PREPARE FRESH.**)

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REAGENTS: (continued)

- D. 100 mM Sodium Citrate Solution (Cit)
(Prepare 10 ml in deionized water using Citric Acid, Trisodium Salt, Dihydrate, Sigma Prod. No. C-7254.)
- E. L-Lactic Dehydrogenase Enzyme Solution (LDH)
(Use L-Lactic Dehydrogenase, Sigma Prod. No. L-2500.²)
- F. Malic Dehydrogenase Enzyme Solution (MDH)
(Use Malic Dehydrogenase, Sigma Prod. No. M-7383.³)
- G. 10 mM Triethanolamine with 0.3 mM Zinc Chloride and 454 mM Ammonium Sulfate Solution (Enz Dil)
(Prepare 100 ml in deionized water using Triethanolamine Hydrochloride, Sigma Prod. No. T-1502, Zinc Chloride, Sigma Prod. No. Z-4875, and Ammonium Sulfate, Sigma Prod. No. A-5132. Adjust to pH 7.6 at 25°C with 1 M NaOH.)
- H. Citrate Lyase Enzyme Solution
(Immediately before use, prepare a solution containing 0.1 - 0.3 unit/ml of Citrate Lyase in cold Reagent G.)

PROCEDURE:

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

Reagent A (Buffer)	28.50
Reagent B (ZnCl ₂)	0.50
Reagent C (β-NADH)	0.50
Reagent D (Cit)	0.20

Mix by inversion and adjust to pH 7.6 at 25°C with 1 M HCl or 1 M NaOH, if necessary.

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

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	2.88	2.88
Reagent E (LDH)	0.01	0.01
Reagent F (MDH)	0.01	0.01

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PROCEDURE: (continued)

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

	<u>Test</u>	<u>Blank</u>
Reagent G (Enz Dil)	-----	0.10
Reagent H (Enzyme Solution)	0.10	-----

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

CALCULATIONS:

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

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

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

UNIT DEFINITION:

One unit will convert 1.0 μ mole of citrate to oxalacetate per minute at pH 7.6 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 96 mM triethanolamine, 0.5 mM zinc chloride, 0.23 mM β -NADH, 0.67 mM sodium citrate, 100 units L-lactic dehydrogenase, 50 units malic dehydrogenase, 15 mM ammonium sulfate and 0.01 - 0.03 unit citrate lyase.

REFERENCES:

Bergmeyer, H.U., Gawehn, K. and Grassl, M. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) Volume

I, Second Edition, 442-443, Academic Press, Inc., New York, NY

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REFERENCES: (continued)

Moellering H. and Gruber, W. (1966) *Analytical Biochemistry* **17**, 369-376

NOTES:

1. The addition of L-Lactic Dehydrogenase ensures that any oxalacetate that is converted to pyruvate is measured. This conversion to pyruvate occurs by either oxalacetate decarboxylase which may be present in citrate lyase or by a nonenzymatic reaction. This is described in Moellering H. and Gruber W. (1966).
2. Contains not less than 10,000 L-Lactic Dehydrogenase units per ml.
3. Contains not less than 5,000 Malic Dehydrogenase units per ml.
4. L-Lactic Dehydrogenase Unit Definition: One unit will reduce 1.0 μ mole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.
5. Malic Dehydrogenase Unit Definition: One unit will convert 1.0 μ mole of oxalacetate and β -NADH to L-malate and β -NAD per minute at pH 7.5 at 25°C.
6. This assay is based on Bergmeyer, H.U. et al. (1974).
7. 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.