

**Enzymatic Assay of ISOCITRATE LYASE
(EC 4.1.3.1)**

PRINCIPLE:

Isocitrate $\xrightarrow{\text{Isocitrate Lyase}}$ Succinate + Glyoxylate

Glyoxylate + Phenylhydrazine \longrightarrow Phenylhydrazine
Glyoxylate

CONDITIONS: T = 30°C, pH = 6.8, A_{324nm}, Light Path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 50 mM Imidazole Buffer, pH 6.8 at 30°C
(Prepare 100 ml in deionized water using Imidazole, Sigma Prod. No. I-0125. Adjust to pH 6.8 at 30°C with 1 M HCl.)
- B. 50 mM Magnesium Chloride Solution (MgCl₂)
(Prepare 10 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)
- C. 10 mM Ethylenediaminetetraacetic Acid Solution (EDTA)
(Prepare 10 ml in deionized water using Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS.)
- D. 40 mM Phenylhydrazine HCl Solution (Phenylhydrazine)
(Prepare 10 ml in deionized water using Phenylhydrazine Hydrochloride, Sigma Prod. No. P-6926.)
- E. 10 mM DL-Isocitric Acid Solution (Isocitrate)
(Prepare 10 ml in deionized water using DL-Isocitric Acid, Trisodium Salt, Sigma Prod. No. I-1252.)
- F. Isocitrate Lyase Enzyme Solution
(Immediately before use, prepare a solution containing 0.05 - 0.07 unit/ml of Isocitrate Lyase in cold Reagent A.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.50	0.50
Reagent B (MgCl ₂)	0.10	0.10
Reagent C (EDTA)	0.10	0.10
Reagent D (Phenylhydrazine)	0.10	0.10
Reagent E (Isocitrate)	0.10	0.10

Mix by inversion and equilibrate to 30°C. Monitor the A_{324nm} until constant using a suitably thermostatted spectrophotometer. Then add:

Reagent F (Enzyme Solution)	0.10	-----
Reagent A (Buffer)	-----	0.10

Immediately mix by inversion and record the increase in A_{324nm} for approximately 5 minutes. Obtain the r A_{324nm}/minute using the maximum linear rate for both the Test and Blank.

CONDITIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{324\text{nm}}/\text{min Test} - r A_{324\text{nm}}/\text{min Blank})(1)(df)}{(16.8)(0.1)}$$

1 = Volume (in milliliter) of assay

df = Dilution factor

16.8 = Millimolar extinction coefficient of Phenylhydrazine

Glyoxylate at 324 nm

0.1 = Volume (in milliliter) of enzyme used

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

UNIT DEFINITION:

One unit catalyzes the formation of 1 μmole of glyoxylate per minute at pH 6.8 at 30°C.

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FINAL ASSAY CONCENTRATION:

In a 1.00 ml reaction mix, the final concentrations are 30 mM imidazole, 5 mM magnesium chloride, 1 mM ethylenediaminetetraacetic acid, 4 mM phenylhydrazine, 1 mM DL-isocitric acid, and 0.005 - 0.007 unit isocitrate lyase.

REFERENCE:

Chell, R.M., Sundaram, T.K., and Wilkinson, A.E., (1978)
Biochemical Journal **173**, 165-177

NOTES:

1. This assay is based on the cited reference.
2. 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.