Enzymatic Assay of ISOCITRATE LYASE
(EC 4.1.3.1)

PRINCIPLE:

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

Glyoxylate + Phenylhydrazine ⟶ 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 ($MgCl_2$)
(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:

	Test	Blank
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 ΔA_{324nm}/minute using the maximum linear rate for both the Test and Blank.

CONDITIONS:

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

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.