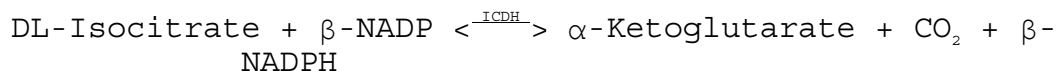


**Enzymatic Assay of ISOCITRIC DEHYDROGENASE (NADP)  
(EC 1.1.1.42)**

**PRINCIPLE:**



Abbreviations:

$\beta$ -NADP =  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate,  
Oxidized Form

$\beta$ -NADPH =  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate,  
Reduced Form

ICDH = Isocitric Dehydrogenase (NADP)

**CONDITIONS:** T = 37°C, pH = 7.4,  $A_{340\text{nm}}$ , Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

- A. 250 mM Glycylglycine Buffer, pH 7.4 at 37°C  
(Prepare 50 ml in deionized water using Gly-Gly, Free Base, Sigma Prod. No. G-1002. Adjust to pH 7.4 at 37°C with 1 M NaOH.)
- B. 6.6 mM DL-Isocitric Acid Solution  
(Prepare 5 ml in Reagent A using DL-Isocitric Acid, Trisodium Salt, Sigma Prod. No. I-1252.)
- C. 20 mM  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate Solution ( $\beta$ -NADP)  
(Prepare 2 ml in deionized water using  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-0505.)
- D. 18 mM Manganese Chloride Solution  
(Prepare 5 ml in deionized water using Manganese Chloride, Tetrahydrate, Sigma Prod. No. M-3634.)
- E. Isocitric Dehydrogenase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.3 - 0.6 unit/ml of Isocitric Dehydrogenase (NADP) in cold Reagent A.)

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**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Deionized Water	1.95	1.95
Reagent A (Buffer)	0.50	0.60
Reagent B (DL-Isocitric Acid)	0.20	0.20
Reagent C ( $\beta$ -NADP)	0.15	0.15
Reagent D (Manganese Chloride)	0.10	0.10

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

Reagent E (Enzyme Solution)	0.10	-----
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Immediately mix by inversion and record the increase in  $A_{340nm}$  for approximately 5 minutes. Obtain  $\Delta A_{340nm}$ /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 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of  $\beta$ -NADPH at 340 nm

0.1 = Volume (in milliliters) of enzyme used

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

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

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**UNIT DEFINITION:**

One unit will convert 1.0  $\mu$ mole of isocitrate to  $\alpha$ -ketoglutarate per minute at pH 7.4 at 37°C.

**FINAL ASSAY CONCENTRATIONS:**

In a 3.00 ml reaction mix, the final concentrations are 67 mM glycylglycine, 0.44 mM DL-isocitric acid, 1.0 mM  $\beta$ -nicotinamide adenine dinucleotide phosphate 0.60 mM manganese chloride, and 0.03 - 0.06 unit isocitric dehydrogenase (NADP).

**REFERENCE:**

Bergmeyer, H.U. (1974) *Methods of Enzymatic Analysis*, Vol. 2, 624-627

**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 information purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.**