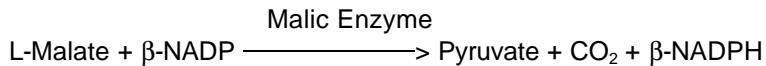


## Enzymatic Assay of MALIC ENZYME (E.C. 1.1.1.40)

### PRINCIPLE:



Abbreviations used:

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

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

**CONDITIONS:** T = 25°C, pH = 7.4,  $A_{340\text{nm}}$ , Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

### REAGENTS:

- A. 100 mM Triethanolamine HCl Buffer, pH 7.4 at 25°C.  
(Prepare 100 ml in deionized water using Triethanolamine, Hydrochloride, Sigma Prod. No. T-1502. Adjust to pH 7.4 at 25°C with 1 M NaOH.)
- B. 100 mM L-Malic Acid Solution (Malic Acid)  
(Prepare 5 ml in deionized water using L(-)Malic Acid, Free Acid, Sigma Prod. No. M-1000.)
- C. 20 mM  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form, Solution (NADP)  
(Prepare 2 ml in deionized water using  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-0505 or  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Stock No. 240-310.)
- D. 20 mM Manganese Chloride Solution ( $\text{MnCl}_2$ )  
(Prepare 25 ml in deionized water using Manganese Chloride, Tetrahydrate, Sigma Prod. No. M-3634.)
- E. Malic Enzyme Solution  
(Immediately before use, prepare a solution containing 0.25 - 0.50 unit/ml of Malic Enzyme in cold deionized water.)

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**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.00	2.00
Reagent B (Malic Acid)	0.10	0.10
Reagent C (NADP)	0.05	0.05
Reagent D (MnCl <sub>2</sub> )	0.75	0.75

Mix by inversion and equilibrate to 25°C. Monitor the A<sub>340nm</sub> until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent E (Enzyme Solution)	0.10	-----
Deionized Water	-----	0.10

Immediately mix by inversion and monitor the increase in A<sub>340nm</sub> for approximately 5-10 minutes. Obtain the ΔA<sub>340nm</sub>/minute using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

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

3 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of β-NADPH 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 convert 1.0 μmole of L-malate and NADP to pyruvate, CO<sub>2</sub> and NADPH per minute at pH 7.4 at 25°C.

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### FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 67 mM triethanolamine, 3.3 mM L-malic acid, 0.3 mM  $\beta$ -nicotinamide adenine dinucleotide phosphate, 5.0 mM manganese chloride and 0.025 - 0.050 unit malic enzyme.

### REFERENCE:

Geer, B.W., Krochko, D., Oliver, M.J., Walker, V.K. and Williamson, J.H. (1980) *Comp. Biochem. Physiol.* 65B, 25-34

### 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.**