SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

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

PRINCIPLE:

$$\text{L-Malate} + \beta\text{-NADP} \xrightarrow{\text{Malic Enzyme}} \text{Pyruvate} + CO_2 + \beta\text{-NADPH}$$

Abbreviations used:
β-NADP = β-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
β-NADPH = β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form

CONDITIONS: T = 25°C, pH = 7.4, A_{340nm}, 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 β-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form, Solution (NADP)
(Prepare 2 ml in deionized water using β-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-0505 or β-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Stock No. 240-310.)

D. 20 mM Manganese Chloride Solution ($MnCl_2$)
(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:

	Test	Blank
Reagent A (Buffer)	2.00	2.00
Reagent B (Malic Acid)	0.10	0.10
Reagent C (NADP)	0.05	0.05
Reagent D ($MnCl_2$)	0.75	0.75

Mix by inversion and equilibrate to 25°C. Monitor the A_{340nm} 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_{340nm} for approximately 5-10 minutes. Obtain the ΔA_{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 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_2 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 β-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.