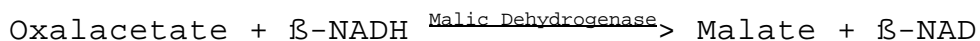
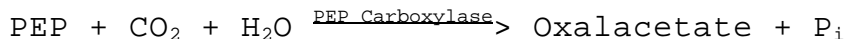


**Enzymatic Assay of PHOSPHO(ENOL)PYRUVATE CARBOXYLASE
(EC 4.1.1.31)**

PRINCIPLE:



Abbreviations used:

PEP = Phospho(enol)pyruvate

PEP Carboxylase = Phospho(enol)pyruvate Carboxylase

P_i = Inorganic Phosphate

β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form

β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: T = 25°C, pH = 8.5, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 110 mM Tris Sulfate Buffer, pH 8.5 at 25°C
(Prepare 100 ml by dissolving Trizma Base, Sigma Prod. No. T-1503 in 110 mM Sulfuric Acid which was prepared in deionized water with Sulfuric Acid, Sigma Prod. No. S-1526. Adjust to pH 8.5 at 25°C with 1 M NaOH.)
- B. 300 mM Magnesium Sulfate Solution (MgSO₄)
(Prepare 10 ml in deionized water using Magnesium Sulfate, Heptahydrate, Sigma Prod. No. M-1880.)
- C. 6 mM β-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β-NADH)
(Dissolve the contents of one 10 mg vial of β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-110, in the appropriate volume of Reagent A. **PREPARE FRESH.**)

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REAGENTS: (continued)

- D. Malic Dehydrogenase Enzyme Solution (MDH)
(Immediately before use, prepare a solution containing 600 units/ml in Reagent A using Malic Dehydrogenase, Sigma Prod. No. M-2634.)
- E. 100 mM Sodium Bicarbonate Solution (Bicarb)
(Prepare 10 ml in deionized water using Sodium Bicarbonate, Sigma Prod. No. S-8875.)
- F. 30 mM Phospho(enol)pyruvate Solution (PEP)
(Prepare 1 ml in Reagent A using Phospho(enol)pyruvate, Monopotassium Salt, Sigma Prod. No. P-7127.)
- G. Dioxane
(Use low peroxide grade)
- H. 300 mM Dithioerythritol Solution (DTE)
(Prepare 1 ml in Reagent A using Dithioerythritol, Sigma Prod. No. D-8255. **PREPARE FRESH.**)
- I. 5.0 mM Magnesium Sulfate Solution (Enzyme Diluent)
(Prepare 20 ml in Reagent A using Magnesium Sulfate, Heptahydrate, Sigma Prod. No. M-1880.)
- J. Phospho(enol)pyruvate Carboxylase Enzyme Solution
(Immediately before use, prepare a solution containing 0.5 - 1 mg/ml of Phospho(enol)pyruvate Carboxylase in Reagent I.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	1.80	1.80
Reagent B (MgSO ₄)	0.10	0.10
Reagent C (β-NADH)	0.10	0.10
Reagent E (Bicarb)	0.30	0.30
Reagent G (Dioxane)	0.30	0.30
Reagent H (DTE)	0.10	0.10

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PROCEDURE: (continued)

Mix by inversion and equilibrate to 25°C. Then add:

	<u>Test</u>	<u>Blank</u>
Reagent D (MDH)	0.01	0.01
Reagent I (Enzyme Diluent)	-----	0.10
Reagent J (Enzyme Solution)	0.10	-----

Mix by inversion and equilibrate to 25°C. Monitor the $A_{340\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent F (PEP)	0.10	0.10
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Immediately mix by inversion and record the decrease in $A_{340\text{nm}}$ for approximately 5 minutes. Obtain the $r A_{340\text{nm}}$ /minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{r A_{340\text{nm}}/\text{min Test} - r A_{340\text{nm}}/\text{min Blank}}{(6.22) (\text{mg enzyme/ml RM})}$$

6.22 = Millimolar extinction coefficient of β -NADH at 340 nm
 RM = Reaction Mix

UNIT DEFINITION:

One unit will form 1.0 μmole of oxalacetate from phospho(enol)pyruvate and CO_2 per minute at pH 8.5 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 2.91 ml reaction mix, the final concentrations are 80 mM Tris sulfate, 10.5 mM magnesium sulfate, 0.21 mM β -NADH, 10 mM sodium bicarbonate, 10% (v/v) dioxane, 10 mM DTE, 1.0 mM PEP, 6 units malic dehydrogenase and 0.05 - 0.1 mg of phospho(enol)pyruvate carboxylase.

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REFERENCE:

Wohl, R.C. and Markus, G. (1972) *Journal of Biological Chemistry* **247**, 5785.

NOTES:

1. Malic Dehydrogenase Unit Definition: One unit will convert 1.0 μ mole of oxalacetate and β -NADH to L-malate and β -NAD per minute at pH 7.5 at 25°C.
2. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.