

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

**PRINCIPLE:**

PEP + CO<sub>2</sub> + H<sub>2</sub>O  $\xrightarrow{\text{PEP Carboxylase}}$  Oxalacetate + P<sub>i</sub>

Oxalacetate + β-NADH  $\xrightarrow{\text{Malic Dehydrogenase}}$  Malate + β-NAD

Abbreviations used:

PEP = Phospho(enol)pyruvate

PEP Carboxylase = Phospho(enol)pyruvate Carboxylase

P<sub>i</sub> = 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<sub>340nm</sub>, 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 (MgSO<sub>4</sub>)  
(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 <sub>4</sub> )	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:

	Test	Blank
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  $\beta$ -NADH at 340 nm

RM = Reaction Mix

**UNIT DEFINITION:**

One unit will form 1.0  $\mu$ mole of oxalacetate from phospho(enol)pyruvate and  $\text{CO}_2$  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  $\beta$ -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  $\mu$ mole of oxalacetate and  $\beta$ -NADH to L-malate and  $\beta$ -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.**