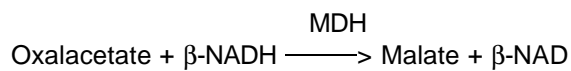
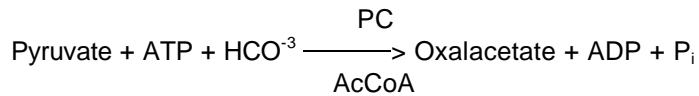


## Enzymatic Assay of PYRUVATE CARBOXYLASE (EC 6.4.1.1)

### PRINCIPLE:



Abbreviations used:

ATP = Adenosine 5'-Triphosphate

PC = Pyruvate Carboxylase

AcCOA = Acetyl Coenzyme A

ADP = Adenosine 5'-Diphosphate

P<sub>i</sub> = Inorganic Phosphate

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

MDH = Malic Dehydrogenase

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

**CONDITIONS:** T = 30°C, pH = 7.8, A<sub>340nm</sub>, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

### REAGENTS:

- A. 135 mM Triethanolamine Buffer with 7 mM Magnesium Sulfate, 9 mM Pyruvic Acid, and 0.15% (w/v) Bovine Serum Albumin, pH 8.0 at 30°C (Substrate)  
(Prepare 100 ml in deionized water using Triethanolamine Hydrochloride, Sigma Prod. No. T-1502, Magnesium Sulfate, Anhydrous, Sigma Prod. No. M-7506, Pyruvic Acid, Sodium Salt, Sigma Prod. No. P-2256, and Albumin, Bovine, Sigma Prod. No. A-6003. Adjust to pH 8.0 at 30°C with 1 M HCl.)
- B. 0.3 mM Acetyl Coenzyme A Solution  
(Prepare 10 ml in deionized water using Acetyl Coenzyme A, Sodium Salt, Sigma Prod. No. A-2056. **PREPARE FRESH.**)

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**REAGENTS:** (continued)

- C. Malic Dehydrogenase Enzyme Solution (AcCoA/MDH)  
(Immediately before use, add 150 units of Malic Dehydrogenase, Sigma Prod. No. M-9004 to 5 ml of Reagent B. Bring the solution to a total volume of 10.0 ml with deionized water.)
  
- D. 100 mM Triethanolamine Buffer with 30 mM Adenosine 5'-Triphosphate and 450 mM Potassium Bicarbonate, pH 8.0 at 30°C (ATP/KHCO<sub>3</sub>)  
(Prepare 10 ml in deionized water using Triethanolamine Hydrochloride, Sigma Prod. No. T-1502, Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394, and Potassium Bicarbonate, Sigma Prod. No. P-9144. Adjust to pH 8.0 at 30°C with 1 M KOH.)
  
- E. 2.6 mM β-Nicotinamide Adenine Dinucleotide, Reduced Form (β-NADH)  
(Prepare 10 ml in deionized water using β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129.)
  
- F. 50 mM Tris HCl Buffer with 50% (v/v) Glycerol, 2 mM Magnesium Acetate, and 1 mM Ethylenediaminetetraacetic Acid, pH 7.4 at 30°C (Enz Dil)  
(Prepare 50 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503, Glycerol, Sigma Prod. No. G-7893, Magnesium Acetate, Tetrahydrate, Sigma Prod. No. M-0631, and Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Hydrate, Sigma Stock No. ED4SS. Adjust to pH 7.4 at 30°C with 1 M HCl.)
  
- G. Pyruvate Carboxylase Enzyme Solution (PC)  
(Immediately before use, prepare a solution containing approximately 30 - 90 units/ml of Pyruvate Carboxylase in cold Reagent F.)

**PROCEDURE:**

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

Reagent A (Substrate)	20.00
Reagent C (AcCoA/MDH)	5.00
Reagent E (β-NADH)	2.50

Mix by swirling. Adjust to pH 7.8 at 30°C if necessary, with either 1 M HCl or 1 M KOH.

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**PROCEDURE:**

Pipette (in milliliters) the following reagents into a suitable container:

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	2.90	2.90
Reagent G (PC)	0.005	-----
Reagent F (Enz Dil)	-----	0.005

Mix by inversion and equilibrate to 30°C. Monitor the  $A_{340\text{nm}}$  until constant using a suitably thermostatted spectrophotometer. Record the  $\Delta A_{340\text{nm}}/\text{minute}$  of the Test.<sup>1</sup> Then add:

Reagent D (ATP/KHCO <sub>3</sub> )	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  $\Delta A_{340\text{nm}}/\text{minute}$  using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340\text{nm}}/\text{min Test} - \Delta A_{340\text{nm}}/\text{min Blank})(3.005)(\text{df})}{(6.22)(0.005)}$$

3.005 = Total volume (in milliliters) of the assay

df = Dilution factor

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

0.005 = Volume (in milliliter) of pyruvate carboxylase used in the assay

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

**UNIT DEFINITION:**

One unit will convert 1.0  $\mu\text{mole}$  of pyruvate and CO<sub>2</sub> to oxalacetate per minute at pH 7.8 at 30°C.

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**FINAL CONCENTRATIONS:**

In a 3.005 ml reaction mix, the final concentrations are 134 mM triethanolamine, 5 mM magnesium sulfate, 7 mM pyruvic acid, 0.12% (w/v) bovine serum albumin, 0.23 mM  $\beta$ -nicotinamide adenine dinucleotide, reduced form, 0.05 mM acetyl coenzyme A, 2.63 units malic dehydrogenase, 1 mM adenosine 5'-triphosphate, 15 mM potassium bicarbonate, 0.05% (v/v) glycerol, 0.002 mM magnesium acetate, 0.001 mM ethylenediaminetetraacetic acid, 0.05 mM Tris, 0.15 - 0.45 unit pyruvate carboxylase.

**REFERENCE:**

Warren, G.B. and Tipton, K.F. (1974) *Biochemical Journal* **139**, 297-310

**NOTES:**

1. Lactic Dehydrogenase is the principle contaminant that may interfere with the assay for pyruvate carboxylase. If the  $\Delta A_{340\text{nm}}/\text{min}$  is not zero, it must be subtracted from the  $\Delta A_{340\text{nm}}/\text{min}$  for the Test after the addition of Reagent D (ATP/KHCO<sub>3</sub>).
2. Malic Dehydrogenase Unit Definition: One unit will convert 1.0  $\mu\text{mole}$  of oxalacetate and  $\beta$ -NADH to L-malate and  $\beta$ -NAD per minute at pH 7.5 at 25°C.
3. This assay is based on the cited reference.
4. 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.**