SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of PYRUVATE CARBOXYLASE (EC 6.4.1.1)

PRINCIPLE:

$$\text{Pyruvate} + \text{ATP} + HCO^{-3} \xrightarrow[\text{AcCoA}]{\text{PC}} \text{Oxalacetate} + \text{ADP} + P_i$$

$$\text{Oxalacetate} + \beta\text{-NADH} \xrightarrow{\text{MDH}} \text{Malate} + \beta\text{-NAD}$$

Abbreviations used:
ATP = Adenosine 5'-Triphosphate
PC = Pyruvate Carboxylase
AcCOA = Acetyl Coenzyme A
ADP = Adenosine 5'-Diphosphate
P_i = 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_{340nm}, 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**.)

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 ($ATP/KHCO_3$)
(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:

	Test	Blank
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_{340nm} until constant using a suitably thermostatted spectrophotometer. Record the ΔA_{340nm}/minute of the Test.[1] Then add:

Reagent D (ATP/$KHCO_3$)	0.10	0.10

Immediately mix by inversion and record the decrease in A_{340nm} for approximately 5 minutes. Obtain the ΔA_{340nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

3.005 = Total volume (in milliliters) of the assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of β-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 μmole of pyruvate and CO_2 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 β-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 ΔA_{340nm}/min is not zero, it must be subtracted from the ΔA_{340nm}/min for the Test after the addition of Reagent D (ATP/$KHCO_3$).

2. Malic Dehydrogenase Unit Definition: One unit will convert 1.0 µmole of oxalacetate and β-NADH to L-malate and β-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.