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Enzymatic Assay of PHOSPHOGLUCOSE ISOMERASE

(EC 5.3.1.9)

PRINCIPLE:

D-Fructose 6-Phosphate $\xrightarrow{PGI}$ D-Glucose 6-Phosphate

D-Glucose 6-Phosphate + β-NADP $\xrightarrow{G\text{-}6\text{-}PDH}$ 6-Phosphogluconate + β-NADPH

Abbreviations used:
PGI = Phosphoglucose Isomerase
β-NADPH = β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form
β-NADP = β-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
G-6-PDH = Glucose-6-Phosphate Dehydrogenase

CONDITIONS: T = 25°C, pH = 7.4, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 250 mM Glycylglycine Buffer, pH 7.4 at 25E

(Prepare 100 ml in deionized water using Glycylglycine, Free Base,. Adjust the pH to 7.4 with 1 M NaOH.)

B. 100 mM D-Fructose 6-Phosphate Solution (F-6-P)

(Prepare 1 ml in deionized water using D-Fructose 6-Phosphate, Disodium.)

C. 20 mM β-Nicotinamide Adenine Dinucleotide Phosphate Solution (NADP)

(Prepare 1 ml in deionized water using β-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt. PREPARE FRESH.)

D. 100 mM Magnesium Chloride Solution (MgCl2)

(Prepare 10 ml in deionized water using Magnesium Chloride, Hexahydrate,.)

E. Glucose-6-Phosphate Dehydrogenase Enzyme Solution (G-6-PDH)

(Immediately before use, prepare a solution containing 50 units/ml of Glucose-6-Phosphate Dehydrogenase, in cold deionized water.)

F. Phosphoglucose Isomerase Enzyme Solution (PGI) (Immediately before use, prepare a solution containing 0.3 - 0.7 unit/ml in cold deionized water.)

PROCEDURE:

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

	Test	Blank
Deionized Water	2.00	2.00
Reagent A (Buffer)	0.50	0.50
Reagent B (F-6-P)	0.10	0.10
Reagent C (NADP)	0.10	0.10
Reagent D ($MgCl_2$)	0.10	0.10
Reagent E (G-6-PDH)	0.10	0.10

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

Reagent F (PGI)	0.10	------
Deionized Water	------	0.10

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

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(3)(df)}{(6.22)(0.1)}$$

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