Determination of the Concentration and Molecular Weight of FRUCTOSE-1,6-DIPHOSPHATE

PROCEDURE:

$F\text{-}1,6\text{-}P_2 \xrightarrow{\text{Aldolase}} GAP + DAP$

$GAP \xrightarrow{\text{TPI}} DAP$

$DAP + \text{ß-NADH} \xrightarrow{\text{a-Glycerophosphate Dehydrogenase}} \text{a-GOP} + \text{ß-NAD}$

Abbreviations used:
$F\text{-}1,6\text{-}P_2$ = Fructose-1,6-Diphosphate
GAP = $_{DL}$-Glyceraldehyde 3-Phosphate
DAP = Dihydroxyacetone Phosphate Dihydroxyacetone
TPI = Triosephosphate Isomerase
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form
a-GOP = $_{L}$-a-Glycerophosphate

CONDITIONS: T = 25°C, pH 7.6, A_{340nm}, Light path = 1 cm

METHOD: Spectrophotometric

REAGENTS:

A. 400 mM Triethanolamine Buffer, pH 7.6 at 25°C (TEA)
(Prepare 100 ml in deionized water using Triethanolamine, Hydrochloride, Prod. No. T-1502. Adjust to pH 7.6 at 25°C with 1 M NaOH.)

B. Fructose-1,6-Diphosphate Solution ($F\text{-}1,6\text{-}P_2$)
(Weigh two samples accurately, approximately 1.5 mg, and dissolve each in 25.0 ml of deionized water.)

C. 4.2 mM ß-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (ß-NADH)
(Prepare 10 ml in Reagent A using ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Prod. No. N-8129.)

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REAGENTS: (continued)

D. a-Glycerophosphate Dehydrogenase-Triosephosphate Isomerase Enzyme Solution (a-GDH/TPI)
(Prepare a solution containing approximately 250 - 500 units/ml of TPI activity with a-Glycerophosphate Dehydrogenase-Triosephosphate Isomerase, Prod. No. G-1881 in cold Reagent A.)

E. Aldolase Enzyme Solution
(Prepare a solution containing approximately 20 units/ml of Aldolase, Type IV from Rabbit Muscle, Prod. No. A-1893, in cold Reagent A.)

PROCEDURE:

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

	Test	Blank
Reagent A (TEA)	1.50	1.60
Reagent B (F-1,6-P_2)	1.00	------
Reagent C (ß-NADH)	0.10	------
Reagent D (a-GDH/TPI)	0.01	0.01
Deionized Water	0.40	1.40

Mix by inversion and obtain the A_{340nm} using a suitable thermostatted spectrophotometer at 25°C. Then add:

Reagent E (Aldolase)	0.01	0.01

Mix by inversion and allow the reaction to proceed for 5 minutes. Upon completion of the reaction record the A_{340nm} and calculate the ?A_{340nm} and apparent molecular weight.

$$\Delta A = A_i \frac{3.01}{3.02} - A_f$$

A_i = Initial absorbance
A_f = Final absorbance

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CALCULATIONS: (continued)

$$micromoles\ F\text{-}1,6\text{-}P_2/weighed\ sample = \frac{\Delta A \times 3.02 \times 25}{(6.22)(2)}$$

3.02 = Total volume of Reaction Mixture
25 = Dilution factor
6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
2 = Number of moles of a-glycerophosphate per mole of fructose-1,6-P_2/weighed sample

$$Apparent\ molecular\ weight = \frac{mg\ sample\ weighed \times 1000}{mmoles\ F\text{-}1,6\text{-}P_2/weighed\ sample}$$

FINAL ASSAY CONCENTRATION:

In a 3.02 ml reaction mix, the final concentrations are 215 mM TEA, 0.14 mM ß-NADH, 0.2 units aldolase and 2.5 - 5.0 units a-GDH/TPI.

REFERENCE:

(1974) *Method of Enzymatic Analysis*, 2nd ed., vol. 3, 1314

NOTES:

1. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.