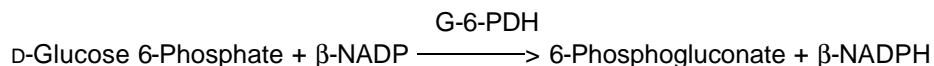
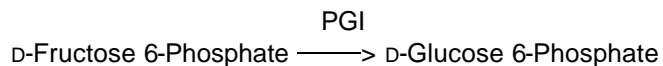


Determination of the Concentration and Molecular Weight of D-FRUCTOSE 6-PHOSPHATE

PRINCIPLE:



Abbreviations used:

PGI = Phosphoglucose Isomerase

β -NADP = β -Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form

G-6-PDH = Glucose-6-Phosphate Dehydrogenase

β -NADPH = β -Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form

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

METHOD: Spectrophotometric Endpoint Determination

REAGENTS:

- A. 300 mM Triethanolamine HCl Buffer, pH 7.6 at 25°C
(Prepare 100 ml in deionized water using Triethanolamine, Hydrochloride, Sigma Prod. No. T-1502. Adjust to pH 7.6 at 25°C with 1 M NaOH.)
- B. 300 mM Magnesium Chloride Solution (MgCl_2)
(Prepare 5 ml in deionized water using Magnesium Chloride, Sterile Filtered Solution, Sigma Prod. No. M-1028.)
- C. 20.0 mM β -Nicotinamide Adenine Dinucleotide Phosphate Solution (β -NADP)
(Prepare 1 ml in deionized water using β -Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-0505. **PREPARE FRESH.**)
- D. 0.25 mM D-Fructose 6-Phosphate Solution (F-6-P)
(Prepare 25 ml in deionized water, using D-Fructose 6-Phosphate, in a volumetric flask.)¹

Determination of the Concentration and Molecular Weight of D-FRUCTOSE 6-PHOSPHATE

REAGENTS: (continued)

- E. Glucose-6-Phosphate Dehydrogenase Enzyme Solution (G-6-PDH)
(Immediately before use, prepare a solution containing 100 units/ml of Glucose-6-Phosphate Dehydrogenase, Sigma Prod. No. G-7877, in cold Reagent A.)
- F. Phosphoglucose Isomerase Enzyme Solution (PGI)
(Immediately before use, prepare a solution containing 100 units/ml of Phosphoglucose Isomerase, Sigma Prod. No. P-5381, in cold Reagent A.)

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 (MgCl ₂)	0.10	0.10
Reagent C (β-NADP)	0.05	0.05
Reagent D (F-6-P)	1.00	-----
Deionized Water	-----	1.00
Reagent E (G-6-PDH)	0.01	0.01

Mix by inversion and equilibrate to 25°C using a suitably thermostatted spectrophotometer. Record the initial $A_{340\text{nm}}$ for both the Test and Blank. Then add:

Reagent F (PGI)	0.01	0.01
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Immediately mix by inversion and allow the reaction to proceed to completion (approximately 10 minutes). Record the final $A_{340\text{nm}}$ for both the Test and Blank.

CALCULATIONS:

$$\Delta A = A_f - A_i$$

A_i = Initial Absorbance

A_f = Final Absorbance

$$\text{Micromoles F-6-P/Weighed sample} = \frac{(\Delta A_{\text{Test}} - \Delta A_{\text{Blank}})(2.97)(\text{df})}{6.22}$$

F-6-P = D-Fructose 6-Phosphate

2.97 = Total volume (in milliliters) of assay

6.22 = Millimolar extinction coefficient of β-NADPH at 340 nm

Determination of the Concentration and Molecular Weight of D-FRUCTOSE 6-PHOSPHATE

CALCULATIONS: (continued)

$$\text{Apparent Molecular Weight} = \frac{\text{mg sample weighed} \times 1000}{\mu\text{moles of F-6-P/weighed sample}}$$

1000 = Conversion from mg to μg
F-6-P = D-Fructose 6-Phosphate

FINAL ASSAY CONCENTRATIONS:

In a 2.97 ml reaction mix, the final concentrations are 184 mM triethanolamine, 10 mM magnesium chloride, 0.34 mM β -nicotinamide adenine dinucleotide phosphate, 1 unit glucose-6-phosphate dehydrogenase, 1 unit phosphoglucose isomerase and varying amounts of fructose-6-phosphate.

REFERENCE:

Lang, G. and Michal, G. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) Volume 3, 2nd ed., 1238-1242

NOTES:

1. When determining the molecular weight for D-Fructose 6-Phosphate, Sigma Prod. Nos. F-0877 and F-6134, dissolve in 1.0 mM Ethylenediaminetetraacetic Acid, using Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Sigma Stock No. ED4SS. Also, correct all weigh-up's for purity water content and salt.
2. Glucose-6-Phosphate Dehydrogenase Unit Definition: One unit will oxidize 1.0 μmole of D-glucose 6-phosphate to 6-phospho-D-gluconate per minute in the presence of β -NADP at pH 7.4 at 25°C.
3. Phosphoglucose Isomerase Unit Definition: One unit will convert 1.0 μmole of D-fructose 6-phosphate to D-glucose 6-phosphate per minute at pH 7.4 at 25°C.
4. This assay is based on the cited reference.
5. 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.