Impurity Assay of a -D-GLUCOSE 1-PHOSPHATE
present in a -D-GALACTOSE 1-PHOSPHATE

PRINCIPLE:

α-D-Glucose 1-Phosphate $\xrightarrow{\text{Phosphoglucomutase}}$ α-D-Glucose 6-Phosphate

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

Abbreviations used:
β-NADP = β-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
β-NADPH = β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced 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 30°C
(Prepare 50 ml in deionized water using Gly-Gly, Free Base, Sigma Prod. No. G-1002. Adjust to pH 7.4 at 25°C with 1 M HCl.)

B. 10 mg/ml Galactose 1-Phosphate Solution, Potassium (Gal-1-P)[1]
(Prepare 2 ml in deionized water using α-D-Galactose 1-Phosphate, Dipotassium Salt, Sigma Prod. No. G-0380.)

C. 20 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 or dissolve the contents of one 30 mg vial of β-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Stock No. 240-330, in the appropriate volume of deionized water. **PREPARE FRESH.**)

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REAGENTS: (continued)

D. 1.2 mM Glucose 1,6-Diphosphate Solution[1] (G 1,6-P)
(Prepare 1 ml in deionized water using α-D-Glucose 1,6-Diphosphate, Cyclohexylammonium Salt, Hydrate Sigma Prod. No. G-7137.)

E. 300 mM Magnesium Chloride Solution ($MgCl_2$)
(Prepare 1 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)

F. Glucose-6-Phosphate Dehydrogenase Solution (G-6-PDH)
(Immediately before use, prepare a solution containing
25 units/ml in cold Reagent A using Glucose-6-Phosphate Dehydrogenase, Sigma Prod. No. G-7877.)

G. Phosphoglucomutase Enzyme Solution (PGLUM)
(Immediately before use, prepare a solution containing 25 units/ml of Phosphoglucomutase, Sigma Prod. No. P-3397, in cold Reagent A.)

PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	1.40	1.40
Deionized Water	------	1.00
Reagent B (Gla 1-P)	1.00	------
Reagent D (G 1,6-P)	0.05	0.05
Reagent C (β-NADP)	0.10	0.10
Reagent E ($MgCl_2$)	0.25	0.25
Reagent F (G-6-PDH)	0.10	0.10

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

Reagent G (PGLUM)	0.10	0.10

Immediately mix by inversion and allow the reaction to proceed until ΔAbs_{340}/minute is ≤ 0.0020, (and this rate is maintained for a minimum of five minutes). Record the final A_{340nm} for both the Test and Blank versus air.

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CALCULATIONS:

ΔA = Af - Ai
Af = Final Absorbance
Ai = Initial Absorbance

$$\text{Micromoles of Glu-1-P/1 ml of Gal-1-P} = \frac{(\Delta A\text{Test} - \Delta A\text{Blank})(3)}{6.22}$$

Glu-1-P = α-D-Glucose-1-Phosphate
Gal-1-P = α-D-Galactose-1-Phosphate
3 = Total volume (in milliliters) of assay
6.22 = Millimolar extinction coefficient of β-NADPH at 340 nm

$$\text{Molar \% Glu-1-P} = \frac{(\mu\text{moles of Glu-1-P/1 ml of Gal-1-P})(100)}{\mu\text{moles of Gal-1-P/1 ml of Gal-1-P}}$$

$$\frac{\mu\text{moles Glu-1-P}}{\text{1.0 ml of Gal-1-P}} = \frac{\text{mgs X 1000}}{\text{Enzymatic MW}}$$

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 117 mM glycylglycine, 0.67 mM β-nicotinamide adenine dinucleotide phosphate, 0.02 mM glucose 1,6-diphosphate, 25 mM magnesium chloride, 2.5 units glucose 6-phosphate dehydrogenase and 2.5 units phosphoglucomutase and varing concentrations of α-D-galactose 1-phosphate.

REFERENCE:

Bergmeyer, H.A., and Michal, G. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) Volume 3, 2nd ed., 1233-1237, Academic Press, Inc., New York, NY

NOTES:

1. If the test material is α-Galactose-1-Phosphate, Barium Salt, Sigma Product No. G-1300, prepare in 30 mM EDTA, using Ethylenediaminetetraacetic Acid, Tetrasodium, Hydrate, Sigma Stock No. ED4SS.

2. Glucose 1,6-Diphosphate is required in order to obtain maximum activity.

3. 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.

4. Phosphoglucomutase Unit Definition: One unit will convert 1.0 μmole of α-D-glucose

1-phosphate to α-D-glucose 6-phosphate per minute at pH 7.4 at 30°C.

NOTES: (continued)

5. This assay is based on the cited references.

6. 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.