

## Impurity Assay of $\alpha$ -D-GLUCOSE 1-PHOSPHATE present in $\alpha$ -D-GALACTOSE 1-PHOSPHATE

### PRINCIPLE:

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

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

Abbreviations used:

$\beta$ -NADP =  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form

$\beta$ -NADPH =  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form

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

**CONDITIONS:** T = 25°C, pH = 7.4,  $A_{340\text{nm}}$ , 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)<sup>1</sup>  
(Prepare 2 ml in deionized water using  $\alpha$ -D-Galactose 1-Phosphate, Dipotassium Salt, Sigma Prod. No. G-0380.)
- C. 20 mM  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate Solution ( $\beta$ -NADP)  
(Prepare 1 ml in deionized water using  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-0505 or dissolve the contents of one 30 mg vial of  $\beta$ -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<sup>1</sup> (G 1,6-P)  
(Prepare 1 ml in deionized water using  $\alpha$ -D-Glucose 1,6-Diphosphate, Cyclohexylammonium Salt, Hydrate Sigma Prod. No. G-7137.)
- E. 300 mM Magnesium Chloride Solution (MgCl<sub>2</sub>)  
(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:

	<u>Test</u>	<u>Blank</u>
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 ( $\beta$ -NADP)	0.10	0.10
Reagent E (MgCl <sub>2</sub> )	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  $A_{340\text{nm}}$  for both the Test and Blank versus air. Then add:

Reagent G (PGLUM)	0.10	0.10
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Immediately mix by inversion and allow the reaction to proceed until  $\Delta\text{Abs}_{340}/\text{minute}$  is  $\leq 0.0020$ , (and this rate is maintained for a minimum of five minutes). Record the final  $A_{340\text{nm}}$  for both the Test and Blank versus air.

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**CALCULATIONS:**

$$\Delta A = A_f - A_i$$

A<sub>f</sub> = Final Absorbance

A<sub>i</sub> = 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 =  $\alpha$ -D-Glucose-1-Phosphate

Gal-1-P =  $\alpha$ -D-Galactose-1-Phosphate

3 = Total volume (in milliliters) of assay

6.22 = Millimolar extinction coefficient of  $\beta$ -NADPH at 340 nm

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

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

**FINAL ASSAY CONCENTRATION:**

In a 3.00 ml reaction mix, the final concentrations are 117 mM glycylglycine, 0.67 mM  $\beta$ -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 varying concentrations of  $\alpha$ -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  $\alpha$ -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  $\mu$ mole of D-glucose 6-phosphate to 6-phospho-D-gluconate per minute in the presence of  $\beta$ -NADP at pH 7.4 at 25°C.
4. Phosphoglucomutase Unit Definition: One unit will convert 1.0  $\mu$ mole of  $\alpha$ -D-glucose

1-phosphate to  $\alpha$ -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.**