SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of SUCROSE PHOSPHORYLASE
(EC 2.4.1.7)

PRINCIPLE:

Sucrose + P_i $\xrightarrow{\text{Sucrose Phosphorylase}}$ Glucose 1-Phosphate + Fructose

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

Glucose 6-Phosphate + β-NAD $\xrightarrow{\text{G-6-PDH}}$ 6-PG + β-NADH

Abbreviations used:
P_i = Inorganic Phosphate
β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form
G-6-PDH = Glucose-6-Phosphate Dehydrogenase
6-PG = 6-Phospho-D-Gluconate
β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form

CONDITIONS: T = 30°C, pH = 7.0, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 60 mM Imidazole Buffer with 12 mM Magnesium Chloride, pH 7.0 at 30°C
(Prepare 200 ml in deionized water using Imidazole, Sigma Prod. No. I-0125, and Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250. Adjust to pH 7.0 at 30°C with 1 M HCl.)

B. 120 mM Sucrose Solution (Sucrose)
(Prepare 100 ml in Reagent A using Sucrose, Sigma Prod. No. S-9378.)

C. 5 mM β-Nicotinamide Adenine Dinucleotide, Oxidized Form Solution (β-NAD)
(Dissolve the contents of one 10 mg vial of β-Nicotinamide Adenine Dinucleotide, Sigma Stock No. 260-110 in the appropriate volume of Reagent B. **PREPARE FRESH**.)

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REAGENTS: (continued)

D. Phosphoglucomutase/Glucose-6-Phosphate Dehydrogenase[1] Solution (PGM/G-6-PDH)
(Prepare a solution containing 20 - 25 units/ml of Phosphoglucomutase, Sigma Prod. No. P-3397, and 20 - 25 units/ml of Glucose-6-Phosphate Dehydrogenase, Sigma Prod. No. G-5760 in Reagent B.)[2]

E. 2 mM α-D-Glucose 1,6-Diphosphate Solution[3] (G1,6DP)
(Prepare 2 ml in Reagent B using α-D-Glucose 1,6-Diphosphate, Potassium Salt, Hydrate, Sigma Sigma Prod. No. G-5750.)

F. 1.5 M Potassium Phosphate Solution, pH 7.0 at 30°C (Phosphate)
(Prepare 10 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.0 at 30°C with 1 M KOH.)

G. Sucrose Phosphorylase Enzyme Solution
(Immediately before use, prepare a solution containing 1.0 - 2.0 units/ml of Sucrose Phosphorylase in cold Reagent B.)

PROCEDURE:

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

	Test	Blank
Reagent B (Sucrose)	2.60	2.70
Reagent C (β-NAD)	0.10	0.10
Reagent D (PGM/G-6-PDH)	0.05	0.05
Reagent E (G1,6DP)	0.05	0.05
Reagent G (Enzyme Solution)	0.10	------

Mix by inversion and equilibrate to 30°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent F (Phosphate)	0.10	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.

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(EC 2.4.1.7)

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-NADH at 340 nm
0.1 = Volume (in milliliters) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will convert 1.0 µmole each of sucrose and phosphate to glucose 1-phosphate and fructose per minute at pH 7.0 at 30°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 58 mM Imidazole, 12 mM magnesium chloride, 116 mM sucrose, 0.2 mM β-nicotinamide adenine dinucleotide, 2.5 units phosphoglucomutase, 2.5 units glucose-6-phosphate dehydrogenase, 0.10 - 0.20 unit sucrose phosphorylase.

REFERENCE:

Silverstein, R., Voet, J., Reed, D., and Abeles, R.H. (1967) *Journal of Biological Chemistry* 242, 1338-1346.

NOTES:

1. G-6-PDH is inhibited by ammonium sulfate.

NOTES: (continued)

2. The two enzymes listed come as ammonium sulfate suspensions. Add 1000 units each of phosphoglucomutase (Sigma Prod. No. P-3397) and glucose-6-phosphate dehydrogenase (Sigma Prod. No. G-5760) to a centrifuge tube. Centrifuge and remove most of the supernatant. Dissolve the pellet in 20 ml of Reagent B. This will give a solution containing 50 units/ml of each enzyme. Sulfate free enzymes of equal specifications can be used without centrifugation; dissolve them in Reagent B.

3. Glucose 1,6-Diphosphate is added to ensure that the glucose 1-phosphate continues to glucose 6-phosphate. It is required in order for the phosphoglucomutase to be optimally active.

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.

5. Glucose-6-Phosphate Dehydrogenase Unit Definition: One unit will oxidize 1.0 µmole of glucose 6-phosphate to 6-phospho-D-gluconate per minute in the presence of NAD at pH 7.8 at 30°C. Either NAD or NADP may be used as the coenzyme. Under optimal conditions, the activity found with NAD is approximately 1.8 times that found with NADP.)

6. This assay is based on the cited reference.

7. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.