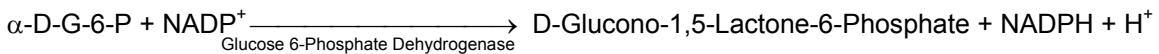
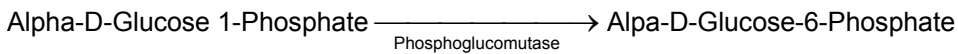
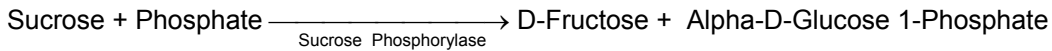


Enzymatic Assay of SUCROSE PHOSPHORYLASE (EC 2.4.1.7)

PRINCIPLE:



ABBREVIATIONS:

NADP⁺ = Beta-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized
NADPH = Beta-Nicotinamide Adenine Dinucleotide Phosphate, Reduced
 α -D-G-6-P = Alpha-D-Glucose-6-Phosphate

METHOD: Spectrophotometric Progress Reaction

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

REAGENTS:

- A. 100 mM Triethanolamine, pH 7.6 at 25°C (TEA)
(Prepare 500 ml in deionized water using 9.286 g to 9.516 g of Triethanolamine Hydrochloride, Sigma Prod. No. T-1502. Adjust pH to 7.6 at 25°C with 1 N NaOH.)
- B. 0.32 M Sucrose (SUCR)
(Prepare 100 ml in Reagent A using 10.96 g to 11.22 g of Sucrose, Minimum 99.5%, Sigma Prod. No. S-9378.)
- C. 100 mM Potassium Phosphate, pH 6.8 at 25°C (PO₄)
(Prepare 100 ml in Reagent A using 0.98 g to 1.03 g of Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379 and 0.488 g to 0.512 g of Potassium Phosphate, Dibasic, Anhydrous, Sigma Prod. No. P-8281.)
- D. 20 mM Ethylenediaminetetraacetic Acid, Tetrasodium (EDTA)
(Prepare 5 ml in Reagent A using 41.62 mg to 45.8 mg of Ethylenediaminetetraacetic Acid, Tetrasodium, Hydrate, Sigma Stock No. ED4SS.)

Sucrose Phosphorylase

REAGENTS(Continued):

- E. 25 mM Beta-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized (NADP⁺)
(Prepare 5 ml in deionized water using 106 mg to 116 mg of Beta-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized, Disodium Salt, Sigma Prod. No. N-0505.)
- F. 0.24 mM Alpha-D-Glucose 1,6- Diphosphate, Cyclohexylammonium Salt (G-1,6-DiPO₄)
(Prepare 0.1 mg / ml in deionized water using Alpha-D-Glucose 1,6-Diphosphate, Cyclohexylammonium Salt, Sigma Prod. No. G-5875. Concentration corrected for water, salt, and solvent content. Prepare fresh.)
- G. 2.0 M Magnesium Chloride (MgCl₂)
(Prepare 5.0 ml in deionized water using 2.033 g to 2.134 g of Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)
- H. Phosphoglucomutase (PgluM)
(Immediately prior to use,prepare a stock solution of 200 Units/ ml cold Reagent A.)
- I. Glucose-6-Phosphate Dehydrogenase (G-6-PDH)
(Immediately prior to use, prepare a stock solution of 200 Units/ ml cold Reagent A)
- J. Sucrose Phosphorylase (Enzyme)
(Immediately prior to use, prepare 5 mg / ml in cold Reagent A. Immediately, dilute to 0.50 Units / ml to 1.50 Units / ml.)

PROCEDURE:

Cocktail¹:

Pipet (in milliliters) the following reagents into a 250 ml, glass beaker:

Reagent B (SUCR)	90.0
Reagent C (PO ₄)	90.0
Reagent D (EDTA)	0.90
Reagent E (NADP ⁺)	3.00
Reagent F (G-1,6-DiPO ₄)	3.00

Reagent G (MgCl₂) 1.50

PROCEDURE(Continued):

Mix and adjust pH to 7.6 with either 0.1N HCl or 0.1 N KOH. at 25°C.

ENZYMATIC

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

	<u>Test-1</u>	<u>Test-2</u>	<u>Test-3</u>	<u>Blank</u>
Cocktail	3.30	3.30	3.30	3.30
Reagent I (G-6-PDH)	0.10	0.10	0.10	0.10
Reagent H (PGluM)	0.10	0.10	0.10	0.10
Reagent A (TEA)	0.010	0.005	-----	0.020

Mix by inverting and equilibrate at 25°C. Then add :

Reagent J (Enzyme)	0.010	0.015	0.020	-----
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Mix by inverting and allow reaction to proceed at 25 °C for seven minutes.
Record the maximum linear rate ($\Delta \text{Abs}_{340\text{nm}}$ / minute) for all test and blank reactions.

CALCULATION:

$$\Delta \text{Abs}_{340\text{nm}} / \text{minute} = \Delta \text{Abs}_{340\text{nm}} / \text{min (Test)} - \Delta \text{Abs}_{340\text{nm}} / \text{minute (Blank)}$$

$$\text{Units / ml} = \frac{\Delta \text{Abs}_{340\text{nm}} / \text{minute} \times 3.52 \times \text{df}}{6.22 \times 0.02}$$

6.22 = Extinction coefficient of Beta- Nicotinamide Adenine Dinucleotide Phosphate,

Reduced at 340 nm

3.52 = Total volume (in milliliters) of Enzymatic Reaction Mixture

0.02 = Volume (in milliliters) of enzyme solution (Reagent J) used in enzymatic reaction

mixture

df = dilution factor of enzyme solution

$$\text{Units/ mg Solid} = \frac{\text{Units / ml}}{\text{Mg solid/ml}}$$

$$\text{Units/ mg Protein} = \frac{\text{Units / ml}}{\text{Mg Protein/ml}}$$

REFERENCES:

1. O. H. Lowry et al. J. Biol. Chem. (1951), **193**, 265.
2. Nakamura, K. et al. J. Ferment. Bioeng., (1998) **85**, 350-353.
3. Biozyme Laboratories International, Ltd. AP-214

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.