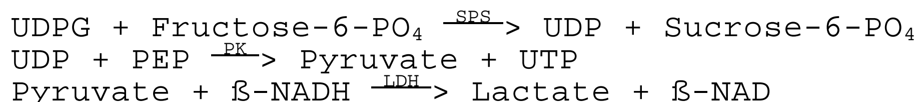
Enzymatic Assay of SUCROSE PHOSPHATE SYNTHETASE (EC 2.4.1.14)

PRINCIPLE:


UDPG + Fructose-6-PO_4 —SPS—> UDP + Sucrose-6-PO_4
UDP + PEP —PK—> Pyruvate + UTP
Pyruvate + ß-NADH —LDH—> Lactate + ß-NAD

Abbreviations used:
UDPG = Uridine 5'-Diphosphoglucose
UDP = Uridine 5'-Diphosphate
SPS = Sucrose Phosphate Synthetase
PEP = Phospho(enol)pyruvate
PK = Pyruvate Kinase
UTP = Uridine 5'-Triphosphate
LDH = Lactic Dehydrogenase
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: T = 37°C, pH = 7.5, A_{340nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 10 mM HEPES NaOH Buffer with 10 mM Magnesium Chloride and 0.004% (w/v) Albumin, pH 7.5 at 37°C
(Prepare 100 ml in deionized water using HEPES, Free Acid, Prod. No. H-3375, Magnesium Chloride, Hexahydrate, Prod. No. M-0250 and Albumin Bovine Serum,
Prod. No. A-4503. Adjust to pH 7.5 at 37°C with 1 M NaOH.)

B. 139 mM Uridine 5'-Diphosphoglucose Solution (UDPG)
(Prepare 1 ml in deionized water using Uridine 5'-Diphosphoglucose, Disodium Salt, Prod. No. U-4625.)

C. 132 mM D-Fructose 6-Phosphate Solution (Fructose-6-PO_4)
(Prepare 1 ml in deionized water using D-Fructose 6-Phosphate, Disodium Salt, Prod. No. F-3627.)

D. PK/LDH Enzyme Suspension[1]

(Use PK/LDH Enzyme Suspension, Stock No. 40-7.)

REAGENTS: (continued)

E. 200 mM Tris HCl Buffer, pH 7.5 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Prod. No. T-1503. Adjust to pH 7.5 at 37°C with 1 M HCl.)

F. 1000 mM Potassium Chloride Solution (KCl)
(Prepare 10 ml in deionized water using Potassium Chloride, Prod. No. P-4504.)

G. 60 mM Magnesium Sulfate Solution ($MgSO_4$)
(Prepare 10 ml in deionized water using Magnesium Sulfate, Heptahydrate, Prod. No. M-1880.)

H. 40 mM Phospho(enol)pyruvate Solution (PEP)
(Prepare 5 ml in deionized water using Phospho(enol)pyruvate, Monopotassium Salt, Prod. No. P-7127.)

I. 0.14 mM ß-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (ß-NADH)
(Prepare the reaction cocktail in a 1 mg vial of ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Stock No. 340-101. The total volume will be 10 ml.)

J. 100 mM Ethylenediaminetetraacetic Acid Solution (EDTA)
(Prepare 10 ml in deionized water using Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Hydrate, Stock No. ED4S.)

K. Sucrose Phosphate Synthetase Enzyme Solution (SPS)
(Immediately before use, prepare a solution containing 1 - 2 units/ml of Sucrose Phosphate Synthetase in cold Reagent A.)

PROCEDURE:

Step 1:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into Reagent I (ß-NADH):

Reagent E (Tris HCl Buffer)	3.40
Reagent F (KCl)	0.50
Reagent G ($MgSO_4$)	1.00
Reagent H (PEP)	0.15
Reagent J (EDTA)	0.05
Reagent D (PK/LDH)	0.15

Deionized Water	4.75

PROCEDURE: (continued)

Mix by inversion. Pipette (in milliliters) the following reagents into suitable cuvettes:

	Test	Blank
Reagent A (HEPES Buffer)	0.50	0.50
Reagent B (UDPG)	0.10	------
Reagent C (Fructose-6-PO_4)	0.40	0.40

Mix by inversion and equilibrate to 37°C. Then add:

	Test	Blank
Reagent K (SPS)	1.00	1.00

Immediately mix by inversion and incubate for exactly 30 minutes at 37°C. Stop the reaction by heating both the Test and Blank solutions for 10 minutes at 100°C. Cool with running tap water. Then add:

	Test	Blank
Reagent B (UDPG)	------	0.10

Step 2:

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

	Test	Blank
Reaction Cocktail	2.90	2.90

Equilibrate to 37°C. Monitor the A_{340nm} until constant using a suitably thermostatted spectrophotometer and record the initial A_{340nm} for both the Test and Blank.[2] Then add:

	Test	Blank
Test Solution	0.10	------
Blank Solution	------	0.10

Immediately mix by inversion and record the decrease in A_{340nm} until complete for approximately 5 minutes. Obtain the final A_{340nm} for both the Test and Blank.

CALCULATIONS:

r A_{340nm} Test = A_{340nm} $Test_{Final}$ - A_{340nm} $Test_{Initial}$

r A_{340nm} Blank = A_{340nm} $Blank_{Final}$ - A_{340nm} $Blank_{Initial}$

Enzymatic Assay of SUCROSE PHOSPHATE SYNTHETASE (EC 2.4.1.14)

CALCULATIONS: (continued)

$$\text{units/mg enzyme} = \frac{A_{340nm}\text{ Test} - A_{340nm}\text{ Blank (3)}}{(6.22)\text{ (mg enzyme/ml RM) (0.1)}}$$

6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
3 = final volume of Step 2
0.1 = Volume from Step 1 used in Step 2
RM = Reaction Mixture

UNIT DEFINITION:

One unit will convert 1.0 µmole each of UDPGlucose and D-fructose 6-phosphate to UDP and sucrose 6-phosphate in 30 minutes at pH 7.5 at 37°C, assayed in a coupled assay system with PK/LDH.

FINAL ASSAY CONCENTRATION:

In a 2 ml reaction mix, the final concentrations are 7.5 mM HEPES, 7.5 mM magnesium chloride, 0.003% bovine serum albumin, 7.0 mM UDPG, 26 mM D-fructose 6-phosphate, and
1 - 2 units sucrose phosphate synthetase.

NOTES:

1. Contains not less than 700 pyruvate kinase units and 1000 lactic dehydrogenase units per ml.

2. The initial absorbance should be between 0.6 - 0.7.

3. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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