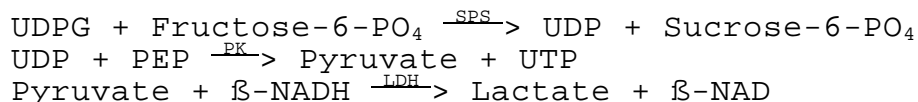


**Enzymatic Assay of SUCROSE PHOSPHATE SYNTHETASE  
(EC 2.4.1.14)**

**PRINCIPLE:**



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

$\beta$ -NADH =  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form

$\beta$ -NAD =  $\beta$ -Nicotinamide Adenine Dinucleotide, Oxidized Form

**CONDITIONS:** T = 37°C, pH = 7.5, A<sub>340nm</sub>, 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<sub>4</sub>)  
(Prepare 1 ml in deionized water using D-Fructose 6-Phosphate, Disodium Salt, Prod. No. F-3627.)
- D. PK/LDH Enzyme Suspension<sup>1</sup>

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

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**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<sub>4</sub>)  
(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 β-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β-NADH)  
(Prepare the reaction cocktail in a 1 mg vial of β-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 (β-NADH):

Reagent E (Tris HCl Buffer)	3.40
Reagent F (KCl)	0.50
Reagent G (MgSO <sub>4</sub> )	1.00
Reagent H (PEP)	0.15
Reagent J (EDTA)	0.05
Reagent D (PK/LDH)	0.15

Deionized Water

4.75

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**PROCEDURE:** (continued)

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

	<u>Test</u>	<u>Blank</u>
Reagent A (HEPES Buffer)	0.50	0.50
Reagent B (UDPG)	0.10	-----
Reagent C (Fructose-6-PO <sub>4</sub> )	0.40	0.40

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

Reagent K (SPS)	1.00	1.00
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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:

Reagent B (UDPG)	-----	0.10
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**Step 2:**

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

Reaction Cocktail	2.90	2.90
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Equilibrate to 37°C. Monitor the A<sub>340nm</sub> until constant using a suitably thermostatted spectrophotometer and record the initial A<sub>340nm</sub> for both the Test and Blank.<sup>2</sup> Then add:

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

Immediately mix by inversion and record the decrease in A<sub>340nm</sub> until complete for approximately 5 minutes. Obtain the final A<sub>340nm</sub> for both the Test and Blank.

**CALCULATIONS:**

$$r_{A_{340nm}} \text{ Test} = A_{340nm} \text{ Test}_{\text{Final}} - A_{340nm} \text{ Test}_{\text{Initial}}$$

$$r_{A_{340nm}} \text{ Blank} = A_{340nm} \text{ Blank}_{\text{Final}} - A_{340nm} \text{ Blank}_{\text{Initial}}$$

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**CALCULATIONS:** (continued)

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

6.22 = Millimolar extinction coefficient of  $\beta$ -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  $\mu$ mole 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.**