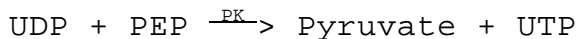
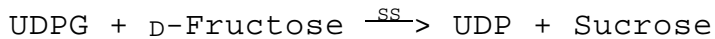


**Enzymatic Assay of SUCROSE SYNTHETASE
(EC 2.4.1.13)**

PRINCIPLE:



Abbreviations used:

UDPG = Uridine 5'-Diphosphoglucose

SS = Sucrose Synthetase

UDP = Uridine 5'-Diphosphate

PEP = Phospho(enol)pyruvate

PK = Pyruvate Kinase

UTP = Uridine 5'-Triphosphate

β -NADH = β -Nicotinamide Adenine Dinucleotide, Reduced Form

LDH = Lactic Dehydrogenase

β -NAD = β -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 Buffer with 10 mM Magnesium Chloride and 0.004% (w/v) Bovine Serum Albumin, pH 7.5 at 37°C (Prepare 100 ml in deionized water using HEPES, Free Acid, Sigma Prod. No. H-3375, Magnesium Chloride, 4.9 M Solution, Sigma Stock No. 104-20, and Albumin Bovine Serum, Sigma Prod. No. A-6003. 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, Sigma Prod. No. U-4625.)
- C. 222 mM D-Fructose Solution (Fructose) (Prepare 1 ml in deionized water using D(-)Fructose, Sigma Prod. No. F-0127.)

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REAGENTS: (continued)

- D. 200 mM Tris HCl Buffer, pH 7.5 at 37°C (Tris HCl)
(Prepare 25 ml in deionized water using Trizma Hydrochloride, Sigma Prod. No. T-3253. Adjust to pH 7.5 at 37°C with 1 M NaOH.)
- E. 1 mM Potassium Chloride Solution (KCl)
(Prepare 5 ml in deionized water using Potassium Chloride, Sigma Prod. No. P-4504.)
- F. 60 mM Magnesium Sulfate Solution (MgSO₄)
(Prepare 10 ml in deionized water using Magnesium Sulfate, Heptahydrate, Sigma Prod. No. M-1880.)
- G. 40 mM Phospho(enol)pyruvate Solution (PEP)
(Prepare 1 ml in deionized water using Phospho(enol)pyruvate, Monopotassium Salt, Sigma Prod. No. P-7127.)
- H. 100 mM Ethylenediaminetetraacetic Acid Solution (EDTA)
(Prepare 1 ml in deionized water using Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Hydrate, Sigma Stock No. ED4S.)
- I. β-Nicotinamide Adenine Dinucleotide, Reduced Form (β-NADH)
(Use β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129.)
- J. PK/LDH Enzymes Suspension¹ (PK/LDH)
(Use PK/LDH Enzymes Suspension, Sigma Stock No. 40-7.)
- K. Sucrose Synthetase Enzyme Solution (SS)
(Immediately before use, prepare a solution containing 1 - 2 units/ml of Sucrose Synthetase in cold Reagent A.)

PROCEDURE:

Step 1:

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

| | <u>Test</u> | <u>Blank</u> |
|--------------------|-------------|--------------|
| Reagent A (Buffer) | 0.50 | 0.50 |
| Reagent B (UDPG) | 0.10 | ----- |

Reagent C (Fructose)

0.40

0.40

**Enzymatic Assay of SUCROSE SYNTHETASE
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PROCEDURE: (continued)

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

| | | |
|----------------|------|------|
| Reagent K (SS) | 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:

| | | |
|------------------|-------|------|
| Reagent B (UDPG) | ----- | 0.10 |
|------------------|-------|------|

Mix by swirling.

Step 2:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

| | | |
|--------------------------------|-------|--|
| Reagent D (Tris HCl) | 20.40 | |
| Reagent E (KCl) | 3.00 | |
| Reagent F (MgSO ₄) | 6.00 | |
| Reagent G (PEP) | 0.90 | |
| Reagent H (EDTA) | 0.30 | |
| Reagent J (PK/LDH) | 0.90 | |
| Deionized Water | 28.50 | |
| Reagent I (β-NADH) | 6 mg | |

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

| | <u>Test</u> | <u>Blank</u> |
|-------------------|-------------|--------------|
| Reaction Cocktail | 2.90 | 2.90 |

Equilibrate to 37°C. Monitor the A_{340nm} until constant (should be between 0.6 to 0.7) using a suitably thermostatted spectrophotometer and record the initial A_{340nm} for both the Test and Blank. Then add:

| | | |
|------------------------------|-------|-------|
| Test Solution (from Step 1) | 0.10 | ----- |
| Blank Solution (from Step 1) | ----- | 0.10 |

Immediately mix by inversion and record the decrease in A_{340nm} until complete (5-10 minutes). Obtain the final A_{340nm} for both the Test and Blank.

**Enzymatic Assay of SUCROSE SYNTHETASE
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CALCULATIONS:

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

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

$$\text{Units/ml enzyme} = \frac{(A_{340\text{nm}} \text{ Test} - A_{340\text{nm}} \text{ Blank})(2)(3)(\text{df})}{(6.22)(1)(0.1)}$$

2 = Volume (in milliliters) of assay (Step 1)

3 = Volume (in milliliters) of assay (Step 2)

df = Dilution factor

6.22 = Millimolar extinction coefficient of β -NADH at 340 nm

1 = Volume (in milliliter) of enzyme used in Step 1

0.1 = Volume (in milliliter) of Test Solution of Step 1 used

in Step 2

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

UNIT DEFINITION:

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

FINAL ASSAY CONCENTRATION:

In a 2.00 ml reaction mix, the final concentrations are 7 mM uridine 5'-diphosphoglucose, 44 mM D-fructose, 7.5 mM HEPES, 7.5 mM magnesium chloride, 0.003% (w/v) bovine serum albumin, and 1 - 2 units sucrose synthetase.

REFERENCE:

Cardini, C.E., LeLoir, L.F., and Chiriboga, J. (1955)
Journal of Biological Chemistry **214**, 149-155

NOTES:

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

2. Pyruvate Kinase Unit Definition: One unit will convert 1.0 μ mole of phospho(enol)pyruvate to pyruvate per minute at pH 7.6 at 37°C.
3. L-Lactic Dehydrogenase Unit Definition: One unit will reduce 1.0 μ mole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.
4. This assay is based on the cited reference.
5. 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.