Enzymatic Assay of SUCROSE SYNTHETASE
(EC 2.4.1.13)

PRINCIPLE:

UDPG + D-Fructose $\xrightarrow{SS}$ UDP + Sucrose

UDP + PEP $\xrightarrow{PK}$ Pyruvate + UTP

Pyruvate + ß-NADH $\xrightarrow{LDH}$ Lactate + ß-NAD

Abbreviations used:
UDPG = Uridine 5'-Diphosphoglucose
SS = Sucrose Synthetase
UDP = Uridine 5'-Diphosphate
PEP = Phospho(enol)pyruvate
PK = Pyruvate Kinase
UTP = Uridine 5'-Triphosphate
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
LDH = Lactic Dehydrogenase
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 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 ($MgSO_4$)
(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. ß-Nicotinamide Adenine Dinucleotide, Reduced Form (ß-NADH)
(Use ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129.)

J. PK/LDH Enzymes Suspension[1] (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:

	Test	Blank
Reagent A (Buffer)	0.50	0.50
Reagent B (UDPG)	0.10	------

Reagent C (Fructose)	0.40	0.40

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 (ß-NADH)	6 mg

Mix by swirling. 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 (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.

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CALCULATIONS:

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

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

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

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 ß-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 µmole 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 µmole 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 µmole 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.