Enzymatic Assay of SUCROSE SYNTHETASE
(EC 2.4.1.13)

PRINCIPLE:

\[
\begin{align*}
\text{UDPG} & \quad + \quad \text{D-Fructose} & \quad \text{SS} & \quad \rightarrow \quad \text{UDP} & \quad + \quad \text{Sucrose} \\
\text{UDP} & \quad + \quad \text{PEP} & \quad \text{PK} & \quad \rightarrow \quad \text{Pyruvate} & \quad + \quad \text{UTP} \\
\text{Pyruvate} & \quad + \quad \beta-\text{NADH} & \quad \text{LDH} & \quad \rightarrow \quad \text{Lactate} & \quad + \quad \beta-\text{NAD}
\end{align*}
\]

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

CONDITIONS:  \(T = 37\, ^\circ C, \quad \text{pH} = 7.5, \quad A_{340\text{nm}}\), 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, Magnesium Chloride, 4.9 M Solution, and Albumin Bovine Serum 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.)

C. 222 mM D-Fructose Solution (Fructose)
(Prepare 1 ml in deionized water using D(-)Fructose.)
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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. 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.)

F. 60 mM Magnesium Sulfate Solution (MgSO₄)  
(Prepare 10 ml in deionized water using Magnesium Sulfate, Heptahydrate.)

G. 40 mM Phospho(enol)pyruvate Solution (PEP)  
(Prepare 1 ml in deionized water using Phospho(enol)pyruvate, Monopotassium Salt.)

H. 100 mM Ethylenediaminetetraacetic Acid Solution (EDTA)  
(Prepare 1 ml in deionized water using Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Hydrate.)

I. β-Nicotinamide Adenine Dinucleotide, Reduced Form  
(β-NADH)  
(Use β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt.)

J. PK/LDH Enzymes Suspension¹ (PK/LDH)  
(Use PK/LDH Enzymes Suspension.)

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:

<table>
<thead>
<tr>
<th>Reagent A (Buffer)</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (UDPG)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>------</td>
</tr>
</tbody>
</table>

¹ PK/LDH Enzymes Suspension is a proprietary product of Creative Enzymes.
<table>
<thead>
<tr>
<th>Reagent C (Fructose)</th>
<th>0.40</th>
<th>0.40</th>
</tr>
</thead>
</table>
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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:

<table>
<thead>
<tr>
<th>Reagent D (Tris HCl)</th>
<th>20.40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent E (KCl)</td>
<td>3.00</td>
</tr>
<tr>
<td>Reagent F (MgSO₄)</td>
<td>6.00</td>
</tr>
<tr>
<td>Reagent G (PEP)</td>
<td>0.90</td>
</tr>
<tr>
<td>Reagent H (EDTA)</td>
<td>0.30</td>
</tr>
<tr>
<td>Reagent J (PK/LDH)</td>
<td>0.90</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>28.50</td>
</tr>
<tr>
<td>Reagent I (β-NADH)</td>
<td>6 mg</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Cocktail</td>
<td>2.90</td>
</tr>
</tbody>
</table>

Equilibrate to 37°C. Monitor the A₃₄₀nm until constant (should be between 0.6 to 0.7) using a suitably thermostatted spectrophotometer and record the initial A₃₄₀nm 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 A₃₄₀nm until complete (5-10 minutes). Obtain the final A₃₄₀nm for both the Test and Blank.
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CALCULATIONS:

\[
\begin{align*}
\text{r } A_{340\text{nm Test}} &= A_{340\text{nm Test Initial}} - A_{340\text{nm Test Final}} \\
\text{r } A_{340\text{nm Blank}} &= A_{340\text{nm Blank Initial}} - A_{340\text{nm Blank Final}} \\
\text{Units/ml enzyme} &= \frac{(A_{340\text{nm Test}} - A_{340\text{nm Blank}})(2)(3)(\text{df})}{(6.22)(1)(0.1)}
\end{align*}
\]

2 = Volume (in milliliters) of assay (Step 1)
3 = Volume (in milliliters) of assay (Step 2)
\(\text{df}\) = Dilution factor
6.22 = Millimolar extinction coefficient of \(\beta\)-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

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

UNIT DEFINITION:

One unit will convert 1.0 \(\mu\)mole each of UDP glucose and \(\alpha\)-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 \(\alpha\)-fructose, 7.5 mM HEPES, 7.5 mM magnesium chloride, 0.003% (w/v) bovine serum albumin, and 1 - 2 units sucrose synthetase.

REFERENCE:


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.

*This procedure is for informational purposes.*