Enzymatic Assay of GLYCOGEN SYNTHASE  
(EC 2.4.1.11)

**PRINCIPLE:**

UDPG + (Glycogen)$_n$ $\overset{Glycogen~Synthase}{\rightarrow}$ UDP + (Glycogen)$_{n+1}$

UDP + PEP $\overset{PK}{\rightarrow}$ UTP + Pyruvate

Pyruvate + $\beta$-NADH $\overset{LDH}{\rightarrow}$ Lactate + $\beta$-NAD

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

**CONDITIONS:**  T = 30°C, pH 8.2, $A_{340nm}$, Light path = 1 cm

**METHOD:**  Spectrophotometric Stop Rate Determination

**REAGENTS:**

A.  500 mM Tris HCl Buffer, pH 8.2 at 30°C (Step 1 Buffer)  
(Prepare 100 ml in deionized water using Trizma Base,  
Prod. No. T-1503.  Adjust to pH 8.2 at 30°C with  
1 M HCl.)

B.  200 mM Tris HCl Buffer, pH 7.5 at 30°C (Step 2 Buffer)  
(Prepare 100 ml in deionized water using Trizma Base,  
Prod. No. T-1503.  Adjust to pH 7.5 at 30°C with  
1 M HCl.)

C.  300 mM Magnesium Chloride Solution (MgCl$_2$)  
(Prepare 25 ml in deionized water using Magnesium  
Chloride, Hexahydrate, Prod. No. M-0250.)

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REAGENTS:  (continued)

D. 100 mM Ethylenediaminetetraacetic Acid Solution (EDTA)  
(Prepare 25 ml in deionized water using  
Ethylenediaminetetraacetic Acid, Tetrasodium Salt,  
Hydrate, Stock No. ED4S.)

E. 100 mM β-Mercaptoethanol Solution (β-ME)  
(Prepare 10 ml in deionized water using  
2-Mercaptoethanol, Prod. No. M-6250.)

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 (MgSO₄)  
(Prepare 10 ml in deionized water using Magnesium  
Sulfate, Anhydrous, Prod. No. M-7506.)

H. 1.0% (w/v) Glycogen Solution (Glycogen)  
(Prepare 200 ml in Reaction Cocktail (Step 1) using  
Glycogen, Prod. No. G-8876.  See "Procedure:  Step 1.")

I. 10 mM D-Glucose 6-Phosphate Solution (G-6P)  
(Prepare 200 ml in Reaction Cocktail (Step 1) using  
D-Glucose 6-Phosphate, Disodium salt, Hydrate,  
Prod. No. G-7250.  See "Procedure:  Step 1.")

J. 40 mM phospho(enol)pyruvate Solution (PEP)  
(Prepare 5 ml in deionized water using  
Phospho(enol)pyruvate, Monopotassium Salt,  
Prod. No. P-7127. See "Procedure:  Step 1.")

K. 3.8 mM Uridine 5'-Diphosphoglucose Solution (UDPG)  
(Prepare 200 ml in Reaction Cocktail (Step 1) using  
Uridine 5'-Diphosphoglucose, Disodium Salt,  
Prod. No. U-4625.  See "Procedure:  Step 1.")

L. 7.1 mM β-Nicotinamide Adenine Dinucleotide Reduced  
Form Solution (β-NADH)  
(Prepare 1 ml in deionized water using β-Nicotinamide  
Adenine Dinucleotide, Reduced Form, Prod. No. N-8129.)

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

N. Glycogen Synthase Enzyme Solution (Glycogen Synth)  
(Immediately before use, prepare a solution containing  
2.0 - 4.0 units/ml of Glycogen Synthase in cold  
deionized water.)
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PROCEDURE:

Step 1:

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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Step 1 Buffer)</td>
<td>20.00</td>
</tr>
<tr>
<td>C (MgCl₂)</td>
<td>8.50</td>
</tr>
<tr>
<td>D (EDTA)</td>
<td>2.00</td>
</tr>
<tr>
<td>E (β-ME)</td>
<td>5.00</td>
</tr>
<tr>
<td>Deionized water</td>
<td>164.50</td>
</tr>
</tbody>
</table>

Mix by swirling and then add (in milligrams) the following reagents to the aforementioned solution in order to make the reaction cocktail:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>K (UDPG)</td>
<td>511.50</td>
</tr>
<tr>
<td>H (Glycogen)</td>
<td>2000.00</td>
</tr>
<tr>
<td>I (G-6P)</td>
<td>564.00</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Cocktail (Step 1)</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>N (Glycogen Synth)</td>
<td>0.10</td>
<td>-------</td>
</tr>
<tr>
<td>Deionized water</td>
<td>------</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Equilibrate to 30°C. Then add:

Immediately mix by inversion and incubate for exactly 5 minutes at 30°C. Stop the reaction by heating both the Test and Blank for 5 minutes at 100°C. Cool with running tap water then transfer both the Blank and Test solutions to Eppendorf tubes and centrifuge.
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PROCEDURE: (continued)

Step 2

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

- Reagent B (Step 2 Buffer) 17.00
- Reagent F (KCl) 2.50
- Reagent G (MgSO\(_4\)) 5.00
- Reagent J (PEP) 0.75
- Reagent D (EDTA) 0.25
- Reagent L (ß-NADH) 1.00
- Deionized water 22.50

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Cocktail (Step 2)</td>
<td>2.80</td>
<td>2.80</td>
</tr>
<tr>
<td>Reagent M (PK/LDH)</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 30°C. Monitor the A\(_{340\text{nm}}\) until constant, using a suitable spectrophotometer. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Supernatant (Step 1)</td>
<td>0.10</td>
<td>------</td>
</tr>
<tr>
<td>Blank Supernatant (Step 2)</td>
<td>------</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the decrease in A\(_{340\text{nm}}\) for approximately 5 minutes. Obtain the final A\(_{340\text{nm}}\) for both the Test and Blank Supernatant.

CALCULATIONS:

\[
\text{r A}_{340\text{nm Test}} = \text{A}_{340\text{nm TestInitial}} - \text{A}_{340\text{nm TestFinal}}
\]

\[
\text{r A}_{340\text{nm Blank}} = \text{A}_{340\text{nm BlankInitial}} - \text{A}_{340\text{nm BlankFinal}}
\]

\[
\begin{align*}
\text{Units/mg enzyme} &= \frac{\text{r A}_{340\text{nm Test}} - \text{r A}_{340\text{nm Blank}} (2.91)}{(5) (6.22) (0.1) (\text{mg enzyme/ml RM})} \\
2.91 &= \text{Final volume of Step 2} \\
0.1 &= \text{Volume form Step 1 used in Step 2} \\
6.22 &= \text{Millimolar extinction coefficient of ß-NADH at 340 nm} \\
5 &= \text{Time of Reaction (in minutes) of Step 1}
\end{align*}
\]
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UNIT DEFINITION:

One unit will catalyze the incorporation of 1.0 µmole of glucose from UDP-glucose into glycogen per minute at pH 8.2 at 30°C, yielding 1.0 µmole of UDP which is measured in a PK/LDH/NADH system.

FINAL ASSAY CONCENTRATION:

In a 3.10 ml reaction mix, the final concentrations are 48 mM Tris, 12.4 MgCl₂, 1.00 mM EDTA, 2.4 mM 2-mercaptoethanol, 3.63 mM UDPG, 9.7 mM glucose 6-phosphate, 0.2 - 0.4 unit glycogen synthase.

REFERENCE:


NOTES:

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

2. 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.