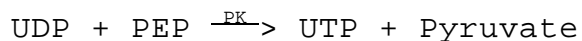
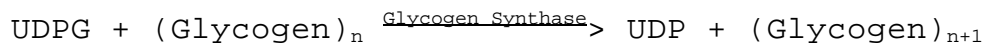


**Enzymatic Assay of GLYCOGEN SYNTHASE  
(EC 2.4.1.11)**

**PRINCIPLE:**



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<sub>340nm</sub>, 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<sub>2</sub>)  
(Prepare 25 ml in deionized water using Magnesium Chloride, Hexahydrate, Prod. No. M-0250.)

**Enzymatic Assay of GLYCOGEN SYNTHASE  
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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  $\beta$ -Mercaptoethanol Solution ( $\beta$ -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 ( $MgSO_4$ )  
(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  $\beta$ -Nicotinamide Adenine Dinucleotide Reduced Form Solution ( $\beta$ -NADH)  
(Prepare 1 ml in deionized water using  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form, Prod. No. N-8129.)
- M. PK/LDH Enzyme Suspension (PK/LDH)  
(Use PK/LDH Enzyme Suspension<sup>1</sup>, 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.)

**Enzymatic Assay of GLYCOGEN SYNTHASE  
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**PROCEDURE:**

Step 1:

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

Reagent A (Step 1 Buffer)	20.00
Reagent C (MgCl <sub>2</sub> )	8.50
Reagent D (EDTA)	2.00
Reagent E (β-ME)	5.00
Deionized water	164.50

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

Reagent K (UDPG)	511.50
Reagent H (Glycogen)	2000.00
Reagent I (G-6P)	564.00

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

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail (Step 1)	3.00	3.00

Equilibrate to 30°C. Then add:

Reagent N (Glycogen Synth)	0.10	-----
Deionized water	-----	0.10

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.

**Enzymatic Assay of GLYCOGEN SYNTHASE  
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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 <sub>4</sub> )	5.00
Reagent J (PEP)	0.75
Reagent D (EDTA)	0.25
Reagent L (β-NADH)	1.00
Deionized water	22.50

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

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail (Step 2)	2.80	2.80
Reagent M (PK/LDH)	0.01	0.01

Mix by inversion and equilibrate to 30°C. Monitor the A<sub>340nm</sub> until constant, using a suitable spectrophotometer. Then add:

Test Supernatant (Step 1)	0.10	-----
Blank Supernatant (Step 2)	-----	0.10

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

**CALCULATIONS:**

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

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

$$\text{Units/mg enzyme} = \frac{r A_{340nm} \text{ Test} - r A_{340nm} \text{ Blank} (2.91)}{(5) (6.22) (0.1) (\text{mg enzyme/ml RM})}$$

2.91 = Final volume of Step 2

0.1 = Volume from Step 1 used in Step 2

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

5 = Time of Reaction (in minutes) of Step 1

**Enzymatic Assay of GLYCOGEN SYNTHASE  
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**UNIT DEFINITION:**

One unit will catalyze the incorporation of 1.0  $\mu$ mole of glucose from UDP-glucose into glycogen per minute at pH 8.2 at 30°C, yielding 1.0  $\mu$ mole 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 MgCl<sub>2</sub>, 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:**

Danforth, W.H. (1965) *Journal of Biological Chemistry* **240**, 588

**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.**