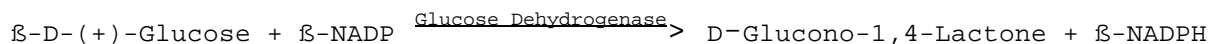


**Enzymatic Assay of GLUCOSE DEHYDROGENASE  
(EC 1.1.1.47)**

**PRINCIPLE:**



Abbreviations used:

$\beta$ -NADP =  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate,  
Oxidized Form

$\beta$ -NADPH =  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate,  
Reduced Form

**CONDITIONS:** T = 37°C, pH 7.0, A<sub>340nm</sub>, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

- A. 50 mM Sodium Phosphate Buffer, pH 7.0 at 37°C  
(Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Monohydrate, Sigma Prod. No. S-9638. Adjust to pH 7.0 at 37°C with 1 M NaOH.)
- B. 500 mM  $\beta$ -D(+)-Glucose Solution (Glucose)  
(Prepare 10 ml in deionized water using  $\beta$ -D(+)-Glucose, Sigma Prod. No. G-5250. **PREPARE FRESH.**)
- C. 4.0 mM  $\beta$ -Nicotinamide Adenine Dinucleotide, Phosphate, Oxidized Form Solution ( $\beta$ -NADP)  
(Prepare by dissolving the contents of one 10 mg vial of  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate, Sodium, Sigma Stock No. 240-310, in the appropriate volume of deionized water. **PREPARE FRESH.**)
- D. Glucose Dehydrogenase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.3 - 0.6 unit/ml of Glucose Dehydrogenase in cold Reagent A.)

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**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.70	0.80
Reagent B (Glucose)	0.10	0.10
Reagent C ( $\beta$ -NADP)	0.10	0.10

Mix by inversion and equilibrate to 37°C. Monitor the  $A_{340\text{nm}}$  until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (Enzyme Solution)	0.10	-----
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Immediately mix by inversion and record the increase in  $A_{340\text{nm}}$  for approximately 5 minutes. Obtain the  $r A_{340\text{nm}}/\text{min}$  using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

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

1 = Volume (in milliliter) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of  $\beta$ -NADPH at 340nm

0.1 = Volume (in milliliter) of enzyme used

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

**UNIT DEFINITION:**

One unit will oxidize 1.0  $\mu\text{mole}$  per minute of  $\beta$ -D-glucose to D-glucono- $\gamma$ -lactone or D-galactose to D-galactonate at pH 7.0 at 37°C in the presence of  $\text{NADP}^+$ .

**FINAL ASSAY CONCENTRATIONS:**

In a 1.00 ml reaction mix, the final concentrations are 40 mM sodium phosphate, 50 mM  $\beta$ -D(+)-glucose, 0.4 mM  $\beta$ -nicotinamide adenine dinucleotide phosphate, and 0.03 - 0.06 unit glucose dehydrogenase.

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**REFERENCE:**

Smith, L.D., Budgen, N., Bungard, S.J., Danson, M.J., and  
Hough, D.W., (1989) *Biochemical Journal* **261**, 973-977

**NOTES:**

1. This assay is based on the cited reference.
2. 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.**